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PRINCIPLES
OF
BACTERIOLOGY

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TO MY PARENTS

Whose endless sacrifices have made my education possible, and whose boundless love has made this a glorious world to live in, this book is dedicated as a token of undying affection and everlasting gratitude, by their son,

THE AUTHOR

PREFACE

While lecturing to the nurses at the St. Vincent's Charity and St. John's Hospitals, of Cleveland, I have been frequently compelled to either modify or add new subject matter to that contained in various textbooks of Bacteriology for Nurses, or face a very unpleasant situation of being told that "we can't find in our book what you have told us." This could be remedied, of course, to some extent, by referring the pupils to standard books written for medical students, but, in the first place, the multiplicity of textbooks is hardly desirable in teaching an elementary subject, and, in the second place, the information sought would usually be found intimately bound with much of what is decidedly "above their heads" and written in too technical a language to be readily accessible.

I have felt, therefore, that it may be worth while to prepare a textbook which represents, with additions, my syllabus of lectures delivered at the above-mentioned training schools, and which, while prepared for the nurses and written in a very simple language, would be fairly complete and would incorporate the latest facts of Bacteriology—such facts as are recognized to be safely out of the zone of polemics and controversies; where the work described does not, as yet, warrant being regarded as an established fact mention is made to that effect.

There are several features to which I have paid special attention, and the book is, perhaps, original to some ex-

tent from the standpoint of the nature of the subject matter included as well as its arrangement.

We begin to realize that it is while studying bacteriology that the rationale and the principles of bacterial prophylaxis are first brought to the nurse's attention, yet but very little information of this nature is usually given and but very little stress is laid on the connection between bacteriology and prophylaxis; it seemed to me that this would be a very appropriate place and a very opportune time to "drive home" the lessons, and for this reason, I have introduced in each chapter dealing with individual microorganisms, a section—"Mode of Infection, Disinfection and Prophylaxis"—as regards the disease caused by particular microorganisms, giving explicit instructions as regards the patient and those who mingle with him, including the nurse, the room and its contents.

With the present need for more and more nurses, both here and "over there," a demand for nurses capable of serving as laboratory assistants and technicians will be felt, and for this reason I have gone into the minute description of the simpler technical procedures, giving complete and detailed directions, taking nothing for granted and leaving nothing to one's imagination.

For this reason I have laid much stress on cultural diagnosis giving much prominence to the several culture media used for the differentiation of the various members of the typhoid-colon-dysentery group. In each chapter a section is provided on Bacteriologic Diagnosis, under which all tests called for in connection with the particular disease are enumerated and complete technic is given, usually describing but one method—the one which has proved most serviceable in my hands.

Sections on Immunotherapy of individual diseases are

provided in each chapter wherever called for, and at the end of each chapter a summary of the most important characteristics is given.

Since the bacteriology is usually taught to the first year pupils, the lecturer on bacteriology usually has to refer to other allied subjects, especially physiology and histology; I have endeavored to make such references as clear as I knew how by giving the full description and derivation of the terms used.

A complete questionnaire is provided at the end of the book to facilitate the review of the subject.

Numerous textbooks, monographs and magazine articles have been consulted—too numerous to be mentioned in their entirety, but whenever special references were made, I have, at all times, endeavored to give full credit where it is due.

In the section on history, I have briefly reviewed the most prominent achievements of the American bacteriologists.

I have tried to incorporate as much of the newer contributions to the theory and practice of bacteriology and serology as possible, including the problems of carriers, Schick's test, Plotz's work on the etiology of typhus, the work of Cole and his coworkers on pneumonia, the promising work of Bull on the serum therapy of the gas bacillus infection, the complement-fixation test for tuberculosis, etc.

My sincere thanks are due to my many friends for various favors conferred upon me in connection with the preparation of this work, and it affords me great pleasure to express my gratitude to Mr. Richard Fluent for the excellent photographs and to Mr. Daniel E. Quilter for his valuable aid in the preparation of the chapters on the "Bacteria in the Soil, Air, Water, and Milk."

Mr. S. Coopersmith was very kind in arranging the different apparatus for the preparation of the photographs.

My sincere thanks are due to my very good friend, Dr. James E. Hallisy, for his kind help in the preparation of the index as well as the reading of the final proof.

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Cleveland, Ohio.

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PRINCIPLES OF BACTERIOLOGY

SECTION I

GENERAL BACTERIOLOGY

CHAPTER I

HISTORICAL

While the existence of minute organisms and their causal relation to some diseases may have been suspected long before the actual proof came—thus the conception of contagion, i. e., transmission of a disease from man to man, is mentioned by Aristotle, Pliny, and also by some mediaeval scientists, it was but natural that their demonstration should follow, above all things, the construction and improvement of magnifying instruments. The honor of having been first to actually see and describe living organisms, too small to be visible to the naked eye, belongs to a Jesuit priest, Kircher (1659), who was followed, a few years later, by the Dutch linen-draper, Leeuwenhoek (1675). There is very little doubt, however, that by far the greater number of the "animalcules" seen by these observers were not bacteria but various protozoa (from Greek *protos* = first, and *zoon* = animal, the lowest form of animal life), as most of their studies were made on water.

The period intervening between the end of the seven-

teenth and the beginning of the nineteenth century did not bring forth any startling discoveries, although some interesting observations were made by Plenciz, who advanced the opinion that each infectious disease was due to a specific microorganism, by Otto Muller and Ehrenberg, the latter having contributed the classification which in its main points, is tenable even today.

The next real advance came in 1838 when Cagniard-Latour, a physicist, showed the living nature of yeasts, found in fermenting substances, a fact corroborated by Schwann; this observation gave rise to much discussion and argument as the general opinion of the nature of fermentation at that time was that it was due to the decomposition of the protein matter, a process held to be essentially a chemical one. This new conception of fermentation was not, however, definitely accepted until Pasteur, the great French scientist, made known his classical studies on the fermentation in wine and beer.

At this time the mooted and extremely important question was that of "spontaneous generation"—were the "animalcules" seen by Kircher and Leeuwenhoek and other microorganisms produced by others of their own kind or could they be produced from different "things," as, for example, mice were produced, according to Pliny, from dirty linen?

Supported by Buffon, Needham, in 1850, claimed that the theory of spontaneous generation was correct, as he could demonstrate that pieces of putrefying material, sealed in flasks, were found, a few days later, to contain enormous numbers of microorganisms; his experiments were repeated by an Italian investigator, Spallanagani, who having subjected the flasks to considerable heat, could not verify Needham's results. Then came the experiments of Schwann, Schultze, Schroeder and Dresch, who

did not find any bacteria in infusion which had been boiled, but still many investigators clung to the "spontaneous generation" until Pasteur did away with it for once and all.

Pasteur showed that filtering air through cotton wool resulted in depositing enormous numbers of microorganisms, and when a single shred of such filter was placed in a sterile fluid, the latter would soon be teeming with bacteria, whereas, if entrance of air into these sterile fluids was prevented such fluids would remain sterile. The theory of spontaneous generation then received its death blow but for one detail—it could not be explained why the application of the same degree of heat did not always result in complete sterility; this last fact remained unexplained until 1870, when Cohn brought to light the existence of bacterial spores and their very high powers of resistance to heat.

Pasteur now turned his attention to the problem of fermentation and not only confirmed the earlier opinion of Cagniard-Latour and others, but pointed out a number of other fermentations, such as those of lactic acid, the decomposition of organic matter by putrefaction, etc. The dependence of the latter upon living agents suggested to the great English surgeon, Lister, the idea that the suppuration of infected wounds was a process identical with that of putrefaction, and working along the lines indicated by Pasteur's work, Lister introduced his antiseptic and aseptic methods and thus achieved immortal fame of having rendered possible modern surgery.

And now we enter upon the era of discoveries of individual causative agents of various infectious diseases.

In 1863, Davaine not only confirmed the earlier observations of Brauell and Pollender (1855) on the anthrax bacillus, but proved that this disease could always be

transmitted by injecting the blood containing these rod-shaped bacilli into other animals. In 1868, Obermeier discovered the spirillum (corkscrew-like bacterium) causing the relapsing fever.

The discovery of the pus-producing bacteria was demonstrated by Koch, Pasteur, Ogston, Rosenbach, and others, about 1882.

In the same year Koch made his epoch-marking discovery of the bacillus of tuberculosis; Koch has also introduced numerous technical procedures which have made possible the modern bacteriology. Very many pathogenic (from Greek *pathos* = disease, *geneo* = to give birth to, disease producing) bacteria were discovered in the succeeding years, until another epoch-marking discovery was made, that of Schaudinn and Hoffmann, in 1905, of the Spirocheta pallida—the organism causing syphilis, one of the most ravaging diseases.

Within the last thirty years a new branch of bacteriology—the science of immunity (resistance to disease) or immunology has been created, chiefly through the work of Pasteur, Metchnikoff, Roux, Ehrlich, Behring, Bordet, and others.

Our own country has contributed much to the science of bacteriology and immunology through the brilliant work of such men as Anderson and Rosenau (the work on anaphylaxis—increased susceptibility to disease, standardization of the diphtheria and the tetanus antitoxins, etc.); Noguchi (the first successful cultivation of Spirocheta pallida, Wassermann test modification, etc.); Cole and his associates (the work on the differentiation of the various types of the pneumococcus and the successful serum treatment of pneumonia caused by Type I); Flexner (the antimeningitic serum); Russell (typhoid vaccination in the United States); Rosenow,

Hektoen, Vaughan (the work on toxins); Park, Plotz (the discovery of the organisms causing the typhus fever); Sewell (the work on the snake venom); Novy, Welch (the discovery of the gas bacillus); Gen. Sternberg (the discovery of the pneumococcus); Bull (who is now doing a most promising work on the serum treatment of gas bacillus infection), and scores of others.

CHAPTER II

GENERAL INFORMATION ABOUT BACTERIA

Bacteria belong to the vegetable kingdom, being minute unicellular organisms.

I. Bacterial Forms

Normally bacteria are of three different kinds so far as their form is concerned:

1. Cocci (plural from Greek *coccos*, meaning kernel), which are round or oval; these usually occur either in clusters or pairs, being called diplococci (meaning double cocci) in the latter case.

2. Bacilli (plural from Latin *bacillus*, meaning "a little rod"), which are straight, rod-shaped organisms, whose ends may be square or convex more frequently) or concave (less frequently).

3. Spirilla (plural from Latin *spirillum*, meaning a coil) which are curved or comma-shaped organisms (see Fig. 1).

Of these three varieties the bacilli are by far the most frequent.

The Size of Bacteria.—The unit of bacterial measurement is a micron (a Greek word meaning small); it is a thousandth part of a millimeter or 1/25000 of an inch, and is usually represented by the Greek letter μ. Cocci usually vary from .1 μ to 2 μ in diameter, the average size being about .1 μ in diameter. Bacilli vary from 1 μ to 3 or 4 μ, some being very large, as, e. g., the an-

thrax bacillus, which is from 5 to 10 μ long. The spirilla vary greatly in size.

The weight of bacteria is, of course, extremely light, the average being about 0.000000001 milligram, that is to say that sixteen hundred million bacilli weigh approximately one milligram; a normal red blood cell is fifty thousand times as heavy as a single colon bacillus.

II. The Structure of the Bacterial Cell

The bacterial cell has an external membrane that is rigid and maintains the shape of bacteria and is called ectoplasm (from the Greek *ecton,* meaning without, and *plasma,* meaning a formed thing).

Fig. 1.—*A,* bacilli; *B,* cocci; *C,* spirilla.

The thickness of this cell wall varies, being thicker in the old than in the young organisms; special stains are required for the demonstration of the ectoplasm.

The substance which comprises the interior of the bacterial cell is called the endoplasm (from Greek *endon,* meaning within), and *plasma,* or cytoplasm (meaning cell substance); this is a clear, colorless, highly refractile substance. Whether or not there is a nucleus (kernel) in the bacterial cell is not yet definitely settled, although the majority of bacteriologists are inclined to believe there is at least a nuclear substance if not a definite nucleus.

Certain bacteria, notably the diphtheria group, exhibit one or more granules which are scattered irregularly in

the endoplasm; they are called Ernst-Babes' or metachromatic (from Greek meaning changing color) granules; nothing definite is known as to their significance.

Capsules.—Quite a few bacteria are surrounded by an envelope or a halo which is called "capsule" (from Latin *capsula,* meaning a little box); the capsules are most frequently present in the organisms (e. g., pneumococcus) when they are first isolated from animal tissues or if

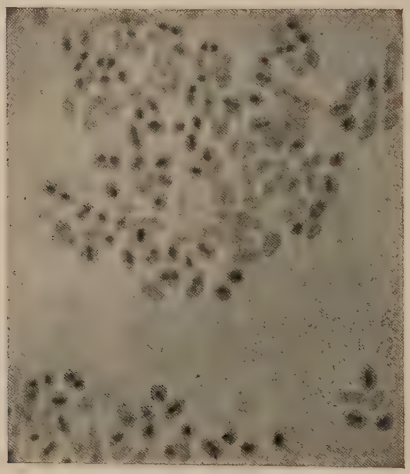


Fig. 2.—Pneumococci, showing capsules. (Mallory and Wright—*Pathological Technic.*)

grown on substances rich in albuminous ingredients, and frequently lose these if grown on ordinary substance. As to the significance of the capsule, the most accepted opinion is that the capsule renders the bacteria more resistant against destruction and thereby more dangerous (virulent, from Latin *vis*, meaning power); the statement that pneumococcus has an especially well defined capsule when freshly isolated from animal tissues and is apt to lose it

when artificially grown, certainly corroborates this view, as pneumococcus needs more resistance, when in the animal body, to exist than in a culture tube. With the exception of pneumococcus mucosus capsulatus (pneumococ-

Fig. 3.—Typhoid bacilli, showing flagella. (Mallory and Wright—*Pathological Technic.*)

cus, Type III) bacteria have to be stained by special methods in order to demonstrate their capsules; this is a very important procedure in making bacteriologic diagnosis in the case of pneumococcus, gas bacilli, etc.

Flagella (from Latin *flagellum*, meaning whip).—All minute objects suspended in fluids are found to be in constant motion; this, however, is not true locomotion, but is irregular and jerky, and is called Brownian movement (after the Scotch investigator, Brown). Some bacteria, however, possess the property of true, independent mobility which is due to the presence of one or more long, delicate, thread-like filaments called flagella (Fig. 3). They have to be stained by special methods.

Spores.—Most of the bacteria when placed in unfavorable environment, that is, when deprived of things es-

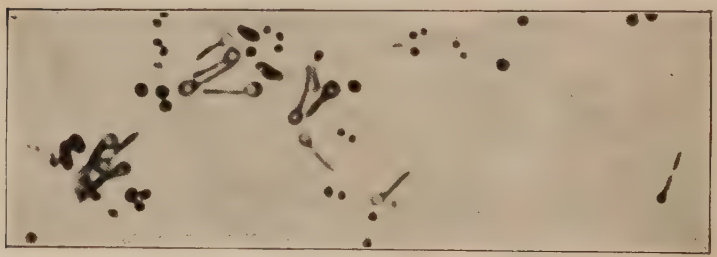


Fig. 4.—Tetanus bacilli, showing spores. (Mallory and Wright—*Pathological Technic.*)

sential to their nutrition or in the presence of harmful substances, die and undergo slow disintegration. But a few bacteria are able to pass into a latent stage of existence similar to hibernation of animals, during which the chemical interchange is at the lowest ebb.

This is accomplished by the bacteria developing within their endoplasm highly refractile, oval bodies called spores (from Greek *sporos*, meaning seed). The spores are highly resistant to heat and disinfectants and thus endow the bacterium with a special resistance against destruction.

Spore formation is not a reproductive process. When

placed in suitable surroundings the spores germinate and the original bacteria reappear. The spore formation is most frequent among those bacteria which live without air (anaerobes), and special staining methods are required for the demonstration of spores.

III. Bacterial Reproduction

The bacteria being minute unicellular asexual cells, the reproduction is a simple mechanical process, the cell having reached its maximum size (which is fairly constant for the species) a slight constriction appears, which deepens until a distinct partition or septum is formed, and the original cell is split into two cells, each being a complete organism, identical with the parent cell. Some of the higher bacteria (e. g., Spirocheta pallida, which causes syphilis) split not transversely but longitudinally (Noguchi). The rate of growth, that is the time elapsing between two successive cleavages, is about 15 to 20 minutes (Fischer), so that a single colon bacillus would yield about 1,500 trillion in a single day.

IV. Constancy of Type

While most of the bacterial properties remain constant, especially the disease-producing (pathogenic, from Greek *pathos,* meaning disease, and *geneo,* meaning to produce) properties, the beginner must remember that under certain conditions one or more characteristics of a given species of the bacterium may be lost, such variation has already been referred to in the connection with the capsule formation. Pasteur succeeded in producing sporeless anthrax bacilli by growing them at 43° C. (instead of 37° C. which is the temperature at which they grow best normally).

V. The Chemical and Physical Properties of the Bacterial Cell

The various chemical constituents of bacteria may be tabulated approximately as follows:

Water	85%
Proteins	10-12%
Fats	1%
Ash	1.5%-2%
Residue	1-1.5%

(Kappes, Nencki and Scheffer.)

This varies, as regards the individual constituents, to some extent; for example, the tubercle bacillus contains less proteins (8 per cent) but much more fats (3.5 to 4 per cent).

The proteins contained in its bacteria are the nucleoproteins, globulins and special proteins. The bacterial ashes are mostly chlorides and phosphates of sodium, potassium, magnesium and calcium.

Like other vegetable and animal cells, the bacterial cell reacts to the pressure which exists between its own protoplasm and the surrounding medium—the so-called osmotic pressure, which governs the exchange of the substances within and without the bacterial cell, this depending upon the permeability of the cell membrane (the ectoplasm), which permits certain substances to enter or leave the bacterial cell.

VI. Nutrition of Bacteria

In order that the bacteria may live and multiply they must have the following substances:

Carbon.—This they may obtain from proteins, fats or carbohydrates.

Oxygen.—This is obtained by the majority of bacteria

directly from the atmosphere in the form of free oxygen. Not all bacteria, however, need oxygen, and some, as will be mentioned later, can not live in its presence.

Nitrogen.—This is taken in most cases, from proteins. There is quite an individual predilection for the special proteins required by different bacteria: the gonococcus, for example, grows best on uncoagulated human blood serum; the influenza bacillus requires hemoglobin (the coloring matter of the red blood cells); while the diphtheria bacillus grows best on the coagulated beef serum. A large number of bacteria may thrive without any protein at all.

Hydrogen.—Hydrogen is obtained largely in combination with water.

Salts.—Salts are absolutely necessary and are chiefly phosphates and chlorides of magnesium, calcium, potassium, and sodium, and iron (in some of the higher bacteria).

VII. Aerobes and Anaerobes

Those bacteria that must have oxygen and can not live without it are called obligatory aerobes.

Those that can not live in the presence of oxygen (e. g., tetanus bacillus) are called obligatory anaerobes. Intermediate between these two extreme groups are those that prefer oxygen but can grow without it—faculative anaerobes—and those that prefer to grow without oxygen but can live in its presence—facultative aerobes.

VIII. Longevity of Bacteria

The duration of bacterial life is unknown, but is, comparatively speaking, brief. Dried spores may not only live, but retain their virulence (invasiveness), for years.

IX. Parasites and Saprophytes

Parasites are those bacteria which live and multiply in the human or animal body, while saprophytes are those which can not hold their own in animal tissues, but are found everywhere in the air, soil, water, and manure. Parasitic bacteria are fastidious as regards their food, temperature, etc., while the saprophytes are easily satisfied and live on simplest media. The main distinction, however, lies in our conception that the parasitic bacteria are those which produce disease, that is, are pathogenic, while the saprophytes live on dead matter and are of greatest value in the world's economy in breaking up the organic matter through the processes of fermentation and putrefaction.

X. Mutual Relations of Bacteria

It is evident that in many cases several different bacteria must live in the same place, or, in other words, the same surrounding must be favorable to several species. This possibility of several bacteria living and multiplying in the presence of each other is spoken of as symbiosis (from Greek *syn,* meaning with, and *bios,* meaning life), as exemplified by diphtheria and streptococci. Symbiosis is not so frequent, however, as the opposite fact—namely, the impossibility of one organism living in the presence of another—a condition which is known as antagonism (from Greek *antagonisma,* meaning struggle); examples of bacterial antagonism are gonococcus and bacillus pyocyaneus, plague bacillus and streptococci, etc.

XI. Relations of Bacteria to Physical Environment

Temperature.—Bacteria, like other living beings, have their minimum (lowest), maximum (highest), and opti-

mum (the best) temperature at which they will live, grow and multiply.

For the large majority of the bacteria the optimum temperature is 37.5° C.; on the other hand, there are numerous bacteria in the water which grow at 20° C.

Individual organisms have their limits, these varying with the environment adopted by them through many generations; for example, the bacillus of the avian (bird) tuberculosis grows at 41° to 42° C. and can not grow at 37.5° C., while the bacillus of human tuberculosis grows best at the latter and will not grow at the former temperature; this difference in the optimum temperature of the two strains of the same organism is due and proportionate to the difference in the normal temperature of human beings and the birds.

By gradual and persistent variation of the temperature it is possible to adapt certain bacteria to grow abundantly at the temperature several degrees higher or lower than their normal optimum temperature, but this is usually accompanied by the loss of certain of their characteristics, as in the case mentioned above—that of anthrax bacillus grown by Pasteur at the temperature of 42° C.—the bacilli finally grew well but lost their property of spore formation.

Some common pathogenic organisms, such as colon bacillus, exhibit a very wide variation of temperature at which they may develop—from 10° C. to 40° C. The saprophytes exhibit an even higher variation.

Ten to fifteen minutes' exposure to a temperature of 55° to 60° C. usually destroys the common pathogenic bacteria which are nonspore-bearing; the spores confer upon bacteria enormous resistance to heat and the spore-bearing bacteria, therefore, are a much more formidable enemy than the other kind.

Low temperatures are much less destructive than the high ones, and in the case of certain bacteria are useful in keeping cultures alive for a long period of time, e. g., pneumococcus and streptococcus.

Moisture.—The presence of water is absolutely necessary for bacterial life. The effects of complete drying vary greatly with the different bacteria; thus gonococcus and cholera organisms die within a few hours while the typhoid and tubercle bacilli may withstand complete drying for two to three months.

Light.—Most of the pathogenic bacteria are inhibited or destroyed by sunlight, although marked variation exists among different bacteria as regards this.

Electricity.—Electric light exerts a distinct bactericidal (bacteria killing) effect when applied in the strength of 800 to 900 candle power for six to nine hours. Roentgen or x-rays do not seem to have any bactericidal effect, while radium has both an inhibitory and bactericidal effect when applied at a distance of a few centimeters for a few hours.

XII. The Biological Activities of Bacteria

While the pathogenic, or disease-producing, properties are of immediate interest to us, we must not lose sight of the fact that these properties are merely a side action, so to speak, compared with other numerous bacterial activities, and that the pathogenic bacteria are the least numerous group of all the bacterial clans. The production of disease is merely incidental to the successful attempt on the part of the bacteria to establish a new domicile in the human or animal body.

The much more important functions of the bacteria are those with which they serve the many important and useful purposes in the world's economy.

It is absolutely no exaggeration to say that without bacteria all reduction of complex organic matter to simple compounds and the chemical interchange between the animal and the vegetable kingdoms could not be carried out and all life on earth would cease, as Hiss and Zinsser so well say (*Textbook of Bacteriology* 1914, p. 41): "Far from being scourges, these minute microorganisms are paramount factors in the great cycle of living matter."

The Katabolic (Breaking Down) Activities of Bacteria.—The katabolic activities of bacteria consist in the fermentation of carbohydrates (sugars and starches) and in the splitting of fats and proteins.

For our entire knowledge of fermentation we are indebted to the genius of Pasteur who was first to explain to us that the fermentation (as well as the splitting of proteins) is due to ferments or enzymes (from Latin and Greek respectively, meaning "leaven"). These are substances produced by a living cell, which produce about a chemical change without entering into the reaction themselves. The ferment is not attached to the end products of the reaction, and is not appreciably diminished during the reaction; in this way the bacterial ferments are like the chemical agents known as katalyzers—e. g., dilute acids, which bring about various chemical reactions and yet do not enter them themselves, as, for example, when a solution of cane sugar brought into contact with dilute solution of sulphuric acid results in the formation of two single sugars in place of a double sugar:

$$C_{12}H_{22}O_{11} + H_2O + H_2SO_4 = C_6H_{12}O_6 + C_6H_{12}O_6 + H_2SO_4.$$

Cane sugar water sulphuric acid = grape sugar fruit sugar sulphuric acid

The proper definition for ferments or enzymes is, therefore, "a substance which hastens a chemical re-

action without itself taking part in it." The best conditions for the ferment action are the presence of moisture, a weakly acid or alkaline reaction, and a temperature ranging from 35° to 45° C.

The presence of protein-splitting, or proteolytic, as they are called, ferments is shown by the power of bacteria to liquefy gelatin, fibrin or coagulated blood serum. Among the important products of bacterial proteolysis are the ptomaines (from Greek *ptoma* meaning a dead body) which are highly poisonous and responsible for ptomaine poisoning.

Certain bacteria produce the so-called coagulating, or "lab" ferments (from Latin *lab*, meaning rennet—a substance found in the stomach, which coagulates milk) which causes coagulation of certain fluids, such as milk, blood, etc. Some bacteria produce fat-splitting ferments (cholera spirillum).

As regards the fermentative properties of bacteria, the most important ferments are those which split the various sugars, cause the production of lactic acid, and the alcoholic fermentation (brought about by both bacteria and yeasts).

A very important class of bacteria is that of denitrifying bacteria—those which reduce the protein decomposition products to nitrates in order that the plants might absorb nitrogen.

The Anabolic (Upbuilding) Activities of Bacteria.—These consist in the property of some soil bacteria to accumulate large amounts of nitrogen from the air and thus to make up for the loss brought about by the absorption of nitrogen by plants (Winogradsky). Even more important than this is the class of bacteria found in the root tubercles of certain plants (leguminosæ), such as beans, peas, etc.; these bacteria not only do not

withdraw nitrogen from the soil but enrich it, and upon this knowledge depends the well-known method of crop alternation used by farmers the world over.

Certain bacteria bring about oxidation of ammonia to nitrites and nitrates; these are called nitrifying bacteria. Some bacteria living in the salt water produce light, and a large number of bacteria (including the pathogenic bacteria) produce various pigments, thus staphylococcus aureus produces a yellow pigment, bacillus pyocyaneus produces a green pigment, while that produced by bacillus prodigiosus is red.

XIII. The Distribution of Bacteria in the Animal Body

While no organ in the animal body, except such structure as nails, is free from invasion from one or another kind of bacterium, still certain organs, in the course of evolution, have come to be preferred by certain bacteria as their domicile, to the exclusion of other organs, and it may be useful to append the following table of bacterial distribution in the body.

Skin.—Staphylococci and Streptococci. Tubercle, leprosy and smegma bacilli. Tetanus, gas, and anthrax bacilli.

Nose and Throat.—Staphylococci, streptococci and pneumococci, diphtheria, influenza and pertussis (whooping cough) organisms. Meningococci and catarrhalis groups. Tubercle bacillus and virus of poliomyelitis (infantile paralysis).

Ear and Eye.—Streptococcus, staphylococcus and pneumococcus groups. Diphtheria and influenza groups. Koch-Weeks and Morax-Axenfeld bacilli. Gonococcus.

Lungs.—Streptococcus and pneumococcus. Tubercle bacillus, staphylococcus, Friedländer's bacillus, influenza and pertussis groups, anthrax and plague bacilli, actinomyces and colon-typhoid group.

Pelvic Organs.—Streptococcus and Staphylococcus groups. Gonococcus and Spirocheta pallida. Tubercle and Smegma bacilli.

Serous Fluids:

1. Cerebrospinal fluid: (a) clear fluid, tubercle bacillus, Spirocheta pallida (syphilis), virus of poliomyelitis. (b) turbid fluid: pneumococcus, streptococcus, meningococcus, B. influenzæ, typhoid-colon group.
2. Pleural and pericardial fluids: (a) clear fluids; tubercle bacillus, (b) turbid fluid: pneumococcus, streptococcus, B. influenza, typhoid, staphylococcus,
3. Peritoneal fluid: Streptococcus group. Colon-typhoid group. Tubercle bacillus.

Blood.—Streptococcus and pneumococcus groups. Typhoid group. Staphylococcus group. Recurrent fever and spirochetæ. Plague bacilli. Friedländer's bacillus (rare).

Intestinal Contents and Feces.—Colon-typhoid group, including the paratyphoids, the dysentery and B. fecalis alcaligenes. Mucosus capsulatus group. Tubercle bacilli, anthrax, tetanus and gas bacilli.

(After Kendall, *Bacteriology*, 1917, p. 106.)

XIV. Bacilli Carriers

Occasionally individuals are met with who, while apparently healthy, harbor pathogenic bacteria in their bodies. While they seem to suffer no ill effects from this, they are a source of danger to those who come in contact with them. Such individuals are called bacilli carriers. In a majority of cases these carriers have had an attack of an infectious disease and have apparently recovered from it, but continue to eliminate the pathogenic bacteria which had caused the disease; some are but temporary carriers, while others may become habitual carriers, never ceasing to eliminate the bacteria. This will be again referred to in the section dealing with infection and immunity, but it is well to call attention, at this time, to the very grave menace of such bacilli carriers.

Many an epidemic, notably of typhoid fever and

diphtheria, has been doubtless brought about by such bacilli carriers; in the case of typhoid fever, the bacilli appear to lodge in the gall bladder or the bile ducts, whence they pass into the intestinal canal and escape in feces. Women are more commonly typhoid carriers than men. Diphtheria carriers are most frequently found among children who often incite a diphtheria epidemic in schools. For this reason all cases of diphtheria and typhoid fever should have repeated examination of throat cultures and feces cultures, respectively, after the patients have apparently recovered. In another section (on Diphtheria) a test will be described, known as Schick's test, which has been devised for the detection of diphtheria carriers.

CHAPTER III

THE DESTRUCTION OF BACTERIA

The destruction of bacteria constitutes what probably is the most glorious chapter of bacteriology—on that alone depended the birth and the development of modern surgery.

Several terms, in this connection, are used, somewhat loosely, and the pupil should, at the outset, clearly understand the different ideas conveyed by them: *by sterilization* is usually meant destruction of bacteria by heat, and is referred to in connection with the boiling of linen, gauze, bandages, infected dressings, instruments, cultures, etc.; *disinfection* usually means destruction of bacteria by the use of chemicals—bichloride of mercury, alcohol, carbolic acid, etc.—and is used in connection with the excreta, urine, feces, or in speaking of destroying bacteria on surgeon's hands or patient's body; fumigation means bacterial destruction by means of certain gases, e. g., formaldehyde, and is used primarily in connection with the buildings or individual rooms; thus we sterilize bandages or the operating room gowns, or dressings; we speak of disinfecting hands with alcohol or the field of the operation—e. g., patient's skin with iodine or of disinfecting the patient's excreta with carbolic acid; and, finally, we fumigate the room with formaldehyde. These different terms refer, however, to the same purpose, accomplished by different means, namely, complete bacterial destruction, or the production of "asepsis"; when a substance does not actually kill the microorganisms, but merely inhibits (prevents) their growth

and multiplication—such substances are spoken of as "antiseptics."

I. Bacterial Destruction by Physical Agents

1. **Drying.**—When complete drying destroys most of the pathogenic bacteria, but the individual bacteria differ greatly from each other in this respect; thus, gonococcus and the bacillus of influenza are destroyed within a few hours, while the tubercle bacillus may live without a trace of moisture for months.

2. **Light.**—Sunlight is a powerful germicide (from Latin meaning life killing), the different bacteria varying from each other in this respect also, the tubercle bacillus dying within two hours; for this reason "where there is sunlight there is no tuberculosis."

3. **Electricity, Radium and Roentgen Rays.**—See the preceding chapter. Briefly stated, x-rays have no influence, while the electricity and radium are both germicidal.

4. **Heat.**—This is the standard and universal method of bacterial destruction.

Dry Heat.—Burning, when we are dealing with objects without value, such as, e. g., the sputum cups, is a very rapid and a sure means of sterilization. Hot air sterilization is carried out in the so-called "hot-air chambers" or sterilizers. (Fig. 5.) This is a double-walled, sheet-iron chamber, with the joints riveted instead of being soldered. The inner case is completely closed, while the outer one has a large opening in the bottom and two small ones at the top; the gas burners —one on each side—are so placed between the two walls that the flame plays directly on the inner case.

This method of sterilization is used primarily for the glassware, and the temperature should be between 150°

and 160° C. maintained for at least one hour. Remember that if combustile articles are sterilized in this sterilizer, the temperature should not exceed 200° C. as cotton is browned at this temperature.

Moist Heat.—*Boiling* may be used for the sterilization of instruments, syringes and other similar objects; vegetative forms (not spores) of bacteria may be destroyed by five minutes' boiling, while in order to de-

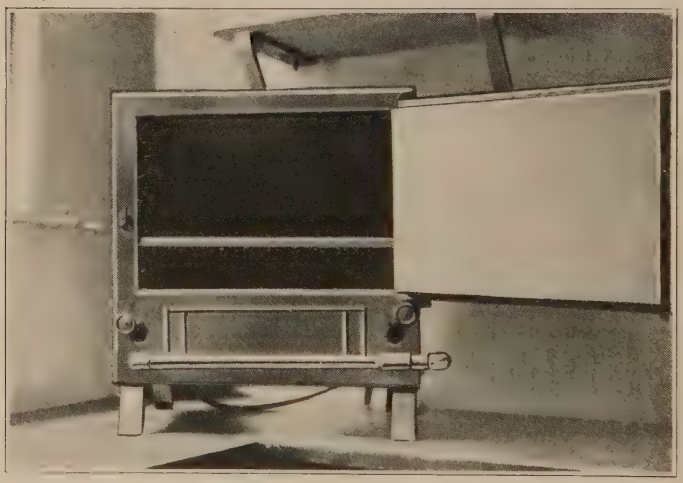


Fig. 5.—Hot-air stèrilizer.

stroy the spores one to two hours' boiling is sufficient.

Live steam is the most practical of the methods of heat sterilization; while it may be improvised by use of almost any simple household makeshifts, such as a double steamer, wash boiler or potato steamer, in the laboratories it is carried out by means of so-called "Arnold" sterilizer (Fig. 6). This consists of a round or rectangular chamber with an outer cylinder well fitting over it, both set in a reservoir about 4 inches high,

which has a shallow double bottom. The reservoir is filled with water which is heated by a gas flame; this rapidly vaporizes the thin layer of water contained in the double bottom of the reservoir, the steam rises rapidly and passes into the inner chamber containing the articles to be sterilized, through its perforated bottom; the steam is condensed under the outer chamber and drops back into the reservoir.

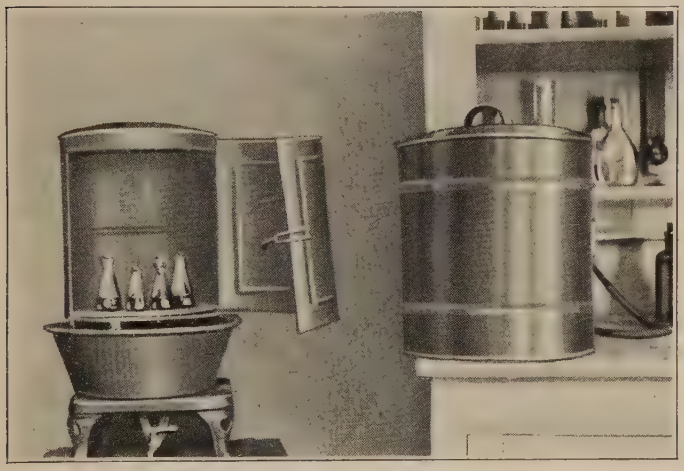


Fig. 6.—The Arnold sterilizer.

An exposure from fifteen to thirty minutes destroys all vegetative forms of bacteria. In order to get rid of spores a "fractional" method of sterilization is used; this consists in exposing the substances to be sterilized (usually culture media) from fifteen to thirty minutes on three successive days; the first exposure destroys the vegetative forms; by next day any spores which may have been present will have developed with the vegetative stage, and are killed by the second ex-

posure; the third exposure makes the sterilization complete. Remember that in noting the time of exposure it is necessary to calculate, not from the time of lighting the flame, but from the time "the steam is up"; that is, from the time the temperature had reached 100° C. When substances to be sterilized can not be subjected to the temperature of 100° C., e. g., culture media containing albuminous materials (because they will coagulate above 60° C.) the principle of fractional sterilization may be adhered to by immersing the objects in a water bath at a temperature of 55° to 60° C. for an hour on five or six consecutive days.

Steam under pressure is the most powerful and certain method of sterilization we possess.

It is used when the objects can not be injured by moisture—operating room gowns, dressings, etc.; in the laboratory this method is used for infected glassware. The apparatus for steam sterilization under pressure is called an "autoclave (Fig. 7). Exposure to steam at the pressure of fifteen pounds for fifteen to twenty minutes will destroy all bacteria and spores.

Two precautions to be borne in mind in connection with the use of autoclave, are: 1. Allow all air to escape from the autoclave before closing the vent. 2. Do not open the door for at least fifteen to twenty minutes after the flame has been turned off, as the sudden relief of pressure thus produced may burn the operator and the stoppers (in tubes or flasks) will "pop out."

II. Bacterial Destruction by Chemical Agents

Many chemicals can either completely destroy the bacteria (bactericides or germicides) or merely inhibit their growth (antiseptics). Just how such injury is done to bacteria is, to a large extent, unknown; some

substances such as strong acids, probably kill bacteria by rapid oxidation; some destroy them by coagulation of the bacterial protoplasm, some enter into chemical combination with the protoplasm and exert a poisonous action, some act by withdrawing water from the bac-

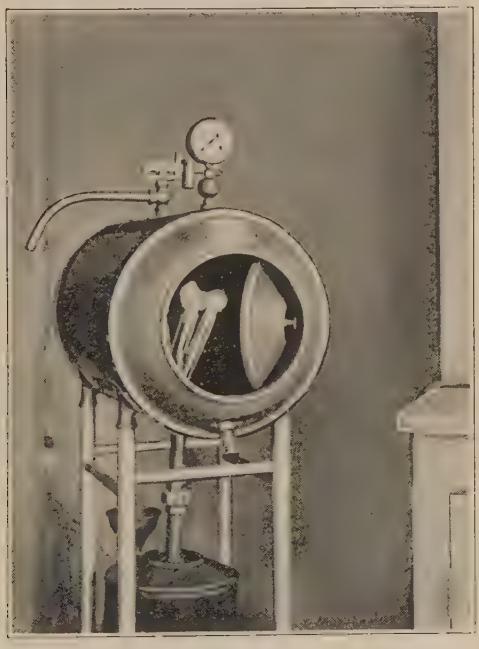


Fig. 7.—The autoclave.

terial cell, etc. Of the many chemicals used for the purposes of disinfection, the following are the most important and the most widely used:

Chloride of Lime or Bleaching Powder.—Chloride of lime is readily soluble in twenty parts of water. In dilution of 1:500 it will destroy the vegetative forms of bacteria in five to ten minutes. See the section on

Practical Disinfection for a brief description of Dakin's solution.

Iodine.—Tincture of iodine (7.5 per cent) has become in the last few years the standard disinfectant; in many hospitals it is the only agent used for disinfecting the field of the operation. It is most effective when freshly prepared; the parts on which it is to be applied should first be swabbed with alcohol and allowed to dry.

Peroxide of Hydrogen.—As the presence of organic matter, such as pus, blood, etc., distinctly diminishes its effects, one should always remove these from wounds before applying the peroxide; it acts upon bacteria by liberating oxygen.

Permanganate of Potassium.—Permanganate of potassium acts, as does the peroxide of hydrogen, and is a powerful germicide.

Bichloride of Mercury (Mercuric Chloride.)—Bichloride of mercury is a powerful germicide; a solution of 1:1000 is commonly used; this will kill the vegetative forms in a few minutes, and in solution of 1:500 will kill spores in a few hours. The addition of 25 per cent alcohol greatly increases the germicidal action of bichloride of mercury. There are certain disadvantages: it is apt to cause local necrosis (destruction) of tissues because of its great affinity for proteins, or injure the kidneys by absorption; it is unreliable for disinfection of feces, sputum, etc., and can not be used for sterilization of instruments. It is used almost exclusively for irrigating wounds and disinfecting the skin.

Silver.—Silver preparations such as silver nitrate (0.1 to 4 per cent), argyrol, protargol, etc., are used principally upon mucous membranes and in the eye (e. g., in the newborn babies to prevent the ophthalmia of the newborn).

Alcohol.—Alcohol is an efficient germicide but only in dilute solutions, the strongest germicidal action being exerted by 50 to 70 per cent solutions; those stronger than this are much less efficient, the absolute alcohol being practically without any effects.

Iodoform.—Iodoform is a weak germicide in itself, but when introduced into wounds, iodine is liberated and it is then a very efficient agent.

Carbolic Acid.—Carbolic acid (phenol) and its derivatives—lysol, cresol, tricresol, creoline, etc., are very efficient germicides, especially the latter; they are not only germicidal but are also poisonous for human tissues; for this reason they should not be applied to wounds in solutions stronger than ¼-1 per cent nor should they be left for any length of time, as gangrene is a common result of their prolonged application. They are the best agents for disinfecting feces, urine, sputum, soiled linen, etc., for which purpose a 5 per cent solution should be used.

Boric Acid.—Boric acid is not a germicide but an antiseptic, that is, it does not destroy the bacteria but prevents their development; it is used upon mucous membranes and in the eye.

Formaldehyde.—Formaldehyde is used either as a gas for fumigation or a 40 per cent solution in water ("formalin"); in the latter form it is an excellent substance to be used for sputum, urine, feces, etc., in solution 2 to 5 per cent. For the use of formaldehyde in fumigation see the next section.

Sulphur.—Sulphur is used chiefly for destroying the insects.

III. Practical Disinfection

Sputum is a very difficult thing to disinfect because the bacteria contained in it are protected by a thick

envelope of mucus; a 5 per cent solution of carbolic acid is very efficient; sputum cups had best be burned; sputum napkins can either be soaked in a 5 per cent solution of carbolic acid or immersed in boiling water for a half hour. Do not use bichloride of mercury as it forms a thick layer of albuminated mercury around the bacteria.

2. **Feces** should be received in a porcelain or metal container and immediately mixed with large amounts of 5 per cent carbolic acid or formalin or 10 per cent chloride of lime, and allowed to remain in contact with these substances for at least one hour before disposing. The soiled parts of the patient should be wiped with a cloth dipped in 2 per cent carbolic acid or cresol, then with water to remove the disinfectant.

3. **Urine** may be disinfected in a manner similar to that in which feces should be disinfected.

4. **Cloth material,** linen, napkins, etc., which had been handled by the patient should be soaked for at least two hours, either in 2 per cent formalin or in 5 per cent carbolic acid, before it is taken out to be boiled —be careful not to remove any infected linen from the patient's room in a dry state.

5. **Bath water** should not be allowed to drain before it had been mixed with an ounce or two of chlorinated lime for at least an hour.

6. **Skin and hands** should be scrubbed with a brush and green soap and then soaked for a few minutes in 1:1000 solution of bichloride of mercury.

A patient recovering from diseases such as smallpox, scarlet fever, measles, and other "eruptive" fevers should receive a bath in 1:1000 solution of bichloride of mercury (taking care not to have the solution enter the patient's mouth, nose, ears, or eyes).

As to the disinfection of the operator's hands, there still exists but little uniformity, some surgeons using the permanganate—oxalic acid, bichloride method (Welch's method)—others using the alcohol-carbolic acid method (Fürbringer) while still others use the bichloride solution only.

As a matter of fact, the belief is gaining ground that the particular antiseptic used plays but an unimportant part, the two main considerations being the use of hot running water, soap and brush for at least 5 minutes, and wearing sterile rubber gloves; during the operation the surgeon usually dips his gloved hands into a basin containing 1:1000 solution of bichloride of mercury.

7. **Surgical instruments, catgut,** etc., are sterilized in different ways in different hospitals. The usual way is to boil the instruments in soda solution.

8. **Thermometers** should be kept in a 5 per cent solution of formalin; when needed, they should be thoroughly rinsed in water.

9. **Rooms, closets,** etc., had best be fumigated by formaldehyde. The best and the simplest method is that of Russell and Evans (Report of State Board of Health of Maine, 1904). For each thousand cubic feet of space ten ounces of formalin and five ounces of potassium permanganate crystals are placed in a two- or three-gallon galvanized iron pail which must have flaring sides because there is much spattering when the reaction between permanganate and formalin takes place; for this reason it is also advisable to place some heavy paper under the pail. Have all closets, drawers, etc., wide open, while all doors, windows, sashes, key-holes, etc., should be tightly plugged—the fumes of formaldehyde being extremely irritating and dangerous. As soon as potassium permanganate and

formalin are mixed, give the pail a good kick whereupon evolution of the formaldehyde gas will take place. Leave the room instantly and lock the door, leaving

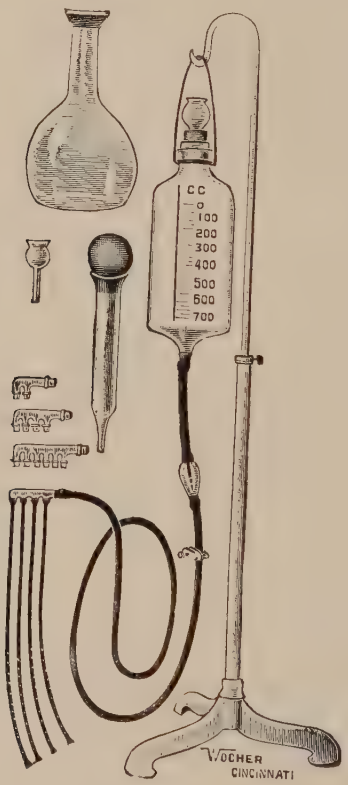


Fig. 8.—Instrument employed in applying Dakin's solution to wounds. It consists of five glass distributing tubes for using single or large number of rubber conducting tubes, the flask is used for mixing the solution and the syringe with rubber bulb is used in testing the permeability of the conducting tubes.

the room undisturbed for at least six hours, at the end of which period all windows should be opened.

10. **Dakin's Solution.**—Within the last two years a

most successful method of treating infected wounds has been devised by Dakin and Carrel (both of the Rockefeller Institute for Medical Research, of New York) and has been extensively applied on battle fields, in industrial plants, hospitals, etc. The preparation of Dakin's solution is somewhat complicated and the original articles by Dakin and Carrel should be consulted; the solution consists of calcium hypochlorated (bleaching powder), sodium carbonate and sodium bicarbonate; these when properly mixed (this means a careful titration of the first mentioned ingredient) cause slow generation of chlorine; the effects of this nascent chlorine differ materially from those ordinary effects of chlorine, since not only the destruction of bacteria in the tissues is ever so much more thorough and rapid but the effect on the dead tissue in the wound is extraordinary—the most infected wounds with dead ragged pieces of tissue become clean and active cicatrization (healing) rapidly takes place. The application of Dakin's solution should be carried out most faithfully by the Carrel method if these unusual results are to be obtained; a special apparatus devised by Carrel must be used, and all the directions given by Dakin and Carrel should be carried out to the letter, without the least deviation.

CHAPTER IV

THE STRUGGLE BETWEEN BACTERIA AND THE BODY INFECTION AND IMMUNITY

I. Infection

By infection we understand more than mere entrance of bacteria to the animal body; an infection means the entrance and successful multiplication of bacteria in the animal body, with the subsequent development of a disease (which is then called infectious disease). That the mere presence of bacteria in the animal body does not mean infection or infectious disease is obvious from the fact that several species of bacteria are constantly present in certain parts of the body; the colon bacilli are always present in the intestines, the bacillus xerosis is very frequently found in the lining of the eyelids (conjunctiva), staphylococci, streptococci, and pneumococci are very often found in normal mouths.

In order that the infection should take place the following conditions should be fulfilled:

1. Bacteria should gain entrance to the body by a path specially adapted to their requirements; thus, certain bacteria invariably attack through the gastrointestinal tract, e. g., the typhoid bacillus and cholera spirillum; some bacteria enter the body only through the skin, e. g., staphylococci, streptococci, tetanus bacillus, etc. These different paths are so necessary for their successful invasion that should the typhoid bacillus (which is a gastrointestinal invader) be rubbed into the skin, or, conversely, should the staphylococcus

(which is a skin invader) be swallowed, no harm would, in all probability, result.

2. Bacteria should invade the body in sufficiently large numbers.

3. They should find an environment which is favorable to their nutritional requirements.

4. The resistance of the individual attacked should be sufficiently weakened to permit the bacteria to develop in his body. (This is discussed fully in a subsequent section on Immunity.)

5. We have mentioned in a preceding chapter that all bacteria are divided into two classes, according to their activities, the saprophytes, those which feed on dead organic matter and are highly useful in maintaining the chemical balance between the animal and the vegetable kingdoms, and the parasites or pathogenic (disease-producing) bacteria which feed on the living body and produce disease; although this division is not absolute, since many saprophytes may become under special conditions pathogenic (for example, they may gain entrance to a gangrenous limb and while feeding on dead organic matter will, nevertheless, cause disease by producing poisonous substances), yet it is convenient to separate the bacteria into these two classes; it is evident that in order that the infection may take place, the bacteria must be pathogenic.

6. Even the pathogenic bacteria differ very much within the same species as to the degree of their power to incite disease; such power is spoken of as virulence; for example, pneumococci kept artificially (that is, in culture media) are much less virulent than those freshly isolated from the animal tissues.

For a successful infection it is necessary, therefore, that the pathogenic bacteria shall possess sufficient virulence.

II. The Infection Proper—Bacterial Poisons

When all of the above conditions have been complied with, we will have an infection, which may be either local or general. If only inflammation of a part or an abscess (pus accumulation) in a certain part results, we speak of this as a local infection; on the other hand from such a local infection—or without it—the bacteria may gain entrance to the lymph and blood vessels and be carried into the circulation and thus be distributed through the entire body—this is called "septicemia" (from Greek *septikos,* meaning putrid and *aima,* meaning blood) or (by the laity) blood poisoning. If the bacteria thus carried to other organs form abscesses there, we speak of this condition as "pyemia" (from Greek *pion,* meaning pus, and *aima,* meaning blood).

Septicemia and pyemia are caused in a large majority of cases by streptococci and staphylococci.

It is evident, however, that mere local injurious effects or blocking of the small blood vessels (capillaries, from Latin meaning hair-like) are not sufficient to account either for severe general effects following infection or for the fact that the same bacteria produce the same injury no matter where they lodge (within their special path of entrance, of course)—in other words it is evident that bacteria produce their effects not mechanically, but chemically, by means of the so-called bacterial poisons, which are called toxins (from Greek *toxicon,* meaning poison).

Those toxins are of three kinds: 1. Exotoxins ("external" poison) which are separable* poisons, secreted by bacteria just as sweat is secreted by the sweat

*I believe the usual terms "soluble" and "insoluble" had better be dismissed, as (in my experience, at least) they usually are a source of confusion to the pupil. "How do the insoluble poisons do any harm if they are insoluble?" is a favorite question.

glands; such separable poison is given off by the bacterium into the surrounding culture medium if it is grown artificially or into the circulation if it lodged in the body. For example, if bacteria are grown in liquid culture, such as broth, and this broth is filtered, it will be found that, although the bacteria will be left on the filter, the liquid substance, which had filtered through, although free from bacteria, will be found to be just as poisonous as the original broth in which the bacteria were grown.

Very few bacteria produce such separable poisons, or exotoxins, the most important bacteria which belong to this class are the diphtheria, the tetanus, and the gas bacilli.

2. Endotoxins ("internal" poisons) are inseparable poisons which are firmly attached to the bacterial cell and are not secreted by it but are liberated only upon the death and disintegration of the bacterium.

The greater number of the pathogenic bacteria produce such poisons, the endotoxins; as, for example, the typhoid and the colon bacilli, the staphylococci and the streptococci, and the cholera spirilla, etc.

3. In addition to these two classes of poisons, there is a third kind, present in all bacterial bodies, after the removal of exotoxins and endotoxins, a certain protein residue, which differs from both of the above in that it is not specific (that is, whether it is derived from the diphtheria or the typhoid bacilli, it will produce the same effects when injected into an animal) and in that its action is very mild, since its injection causes only mild local inflammations or abscesses.

Besides the difference in the mode of production the exotoxins differ from the endotoxins in that the former are destroyed by lower temperature than the latter.

Now that we know that the bacteria act not by their

mere presence, but through the production of different poisons, the next question which suggests itself is: How do the bacterial poisons act?

It is one of the most widely accepted facts in the whole domain of bacteriology that the bacterial poisons, whether exotoxins or endotoxins, have a more or less definite selective chemical action on special tissues and organs, thus the exotoxins of the tetanus bacillus acts specifically on the nervous tissue, the endotoxin of the streptococcus, and staphylococcus act on the red blood cells, and so forth.

The special selective action, or, to borrow the term from chemistry, affinity, which seems to exist between certain bacteria and certain tissues, depends upon the physical and chemical ability of the poisons to enter into union with the tissue cells. The famous experiment brought forth as a proof of this contention is that of Wassermann and Takaki; viz., if brain tissue is allowed to remain in the solution of the tetanus exotoxin, and is then removed, the remaining fluid is free from any poisonous effects, while the injection of the brain tissue into an animal will result in the production of tetanus, thus showing that the brain tissue had actually absorbed the tetanus exotoxin from the solution.

III. Immunity (from Latin "immunis," meaning safe)

When we stop to consider that so many people harbor enormous numbers of bacteria while only a small number of individuals actually develop infection and a still much smaller number dies of infections, we are brought face to face with the fact that there is some peculiarity about some people that, under similar circumstances, permits them to escape the infection while

others succumb to it, or permits them to recover from infections while others die from the same infection.

This "something" or "vitality," as we frequently speak of it, is that peculiarity in the animal body which is spoken of as immunity, and we may define it as a lack of susceptibility to bacterial disease (which prevents us from contracting it) and a power of resistance which enables us to recover from bacterial disease (if the latter develops).

It is evident, therefore, that the term "immunity" expresses the idea that the resistance is not absolute since in some people it is so strong as to prevent them from infections, while in others it is only strong enough to secure for them a recovery from them, while in still others, it is not even strong enough to do that, and such individuals die.

Classification and Varieties of Immunity

Since the term "immunity" itself is only relative it is evident that the classification of its various types will also be, at best, only relative, yet it is convenient to consider the immunity as being of several distinct types.

1. **Natural, inherited or congenital immunity** is that which exists in entire races, species or individuals at their very birth and is just as much one of their characteristics as their anatomic make-up. For example, influenza and leprosy never occur—either spontaneously or through artificial inoculation—among animals. Among the different animal species there are great differences in resistance; for example, rats and dogs are remarkably immune to anthrax and the common fowl can not be infected with tetanus.

Furthermore, within one and the same species the

different races show different degrees of resistance to the same infection; our own domestic sheep are much less resistant to anthrax than the Algerian sheep; among the human beings we know that while the American Indians and negroes are much more susceptible to tuberculosis than the white men, the latter are much more susceptible to yellow fever than are the negroes. In connection with this racial immunity the fact must not be lost sight of that we are also dealing with the different hygiene conditions and customs which doubtless play a part in this question.

Finally within the same race there exists great variation in susceptibility and resistance among the individual members, as seen, for example, in schools, when during an outbreak of an epidemic only a few pupils contract the infection.

2. **Acquired immunity** is one which does not exist in individuals at birth, but which develops in them either as a result of having had an attack of an infectious disease or is brought about by artificial means.

It is well known that many infectious diseases occur but once in the same individual, one attack usually conferring a lasting immunity against subsequent attacks; the following table from Zinsser's *Infection and Resistance* (published by the MacMillan Co., 1914, p. 60) may be referred to in this connection.

Infectious diseases in which one attack usually confers lasting immunity:	Infectious diseases in which one attack does *not* confer lasting immunity:
Plague	Pyogenic infections.
Typhoid fever (Second attacks rare—in 2.4 per cent cases)	Gonorrhea
Cholera	Pneumonia
Smallpox, Chickenpox, Scarlet fever } Second attack very rare	Influenza
Measles—Secondary attacks quite rare	Dengue fever
Yellow fever	Diphtheria
Typhus fever	Recurrent fever
Syphilis—Reinfection rare	Tetanus
Mumps—Secondary attack rare	Erysipelas
	Beriberi
	Malaria
	Tuberculosis

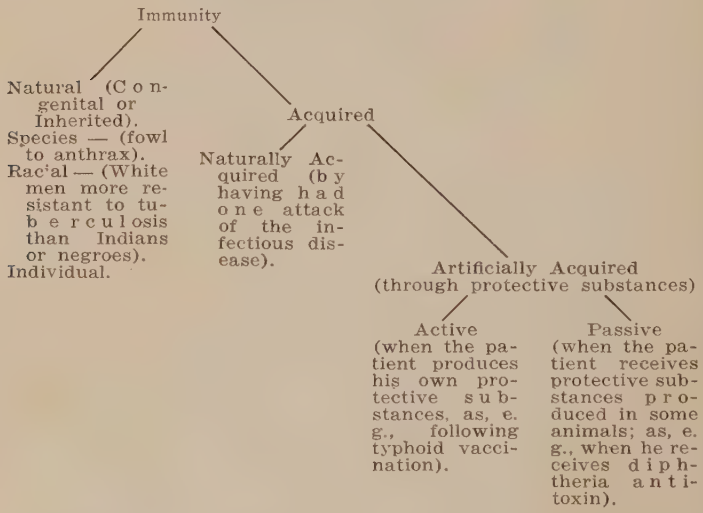
Such immunity—not congenital but brought about by having had one attack of an infectious disease—is called "naturally acquired immunity."

If it is acquired by artificial means, it is spoken of as "artificial acquired immunity," and may be either "active or passive."

Active immunity is that variety of the artificially acquired immunity in which the patient receives an injection of dead bacteria (as in typhoid vaccination) or of the matter from a sore (as in smallpox vaccination); in such cases the patient is really made to undergo a very mild attack of the disease against which he is vaccinated, with the purpose of producing protective substances which will render him insusceptible to the disease proper; this immunity is called "active" because the patient takes an active part in the production of immunity—he himself elaborates the protective substances.

Passive immunity is that variety of the artificially acquired immunity in which the patient receives, in the injection, not the dead bacteria or their poisons, but the protective substances themselves; for example, in the case of diphtheria, a horse is injected with the diphtheria exotoxin (in gradually increasing doses) until he has a vast number of protective substances, then his blood (in reality the blood serum) containing these protective substances is injected into a patient suffering from diphtheria; since the patient did not take an active part in the elaboration of the protective substances, this variety of immunity is called "passive" (on the other hand, the immunity produced in the horse by the injection of the diphtheria exotoxin is, of course, active, since the animal had elaborated its own protective substances).

These varieties of immunity are graphically represented in the following diagram.


Immunity
Natural (Congenital or Inherited).
Species — (fowl to anthrax).
Racial — (White men more resistant to tuberculosis than Indians or negroes).
Individual.
Acquired
Naturally Acquired (by having had one attack of the infectious disease).
Artificially Acquired (through protective substances)
Active (when the patient produces his own protective substances, as, e. g., following typhoid vaccination).
Passive (when the patient receives protective substances produced in some animals; as, e. g., when he receives diphtheria antitoxin).

To make this still clearer let us take as an example the typhoid fever and various types of immunity which may exist against it: if a person is born immune to it, this will be natural immunity; if he develops immunity after birth, this is acquired immunity; if immunity was the result of this person's having had an attack of typhoid fever, we will call this naturally acquired immunity; if the person developed it after he had received an injection of dead typhoid bacilli, he has the active artificial immunity; but if he had been injected with the blood serum of an animal which had received dead typhoid bacilli, then his would be the passive artificial immunity.

IV. Protective Substance or Immune Bodies

Now that we have considered the various types of immunity we have to study just what the protective substances, which constitute the immunity, are:

After all the conditions necessary for a successful infection have been satisfied (see the section on Infection) and the patient has become a victim of an infectious disease, his organism makes an attempt to overcome it by producing the substances which will destroy the infective agent, i. e., the bacteria and their poisons.

These protective substances are called immune bodies or antibodies; just where in the body they are produced (that is, in what particular organ or organs) is unknown, but they are always found in the blood serum.

A few words as to the blood: the blood of the living person is always liquid and consists of a fluid part called plasma; in this plasma are three kinds of blood cells: the red cells (erythrocytes, from Greek *erytros*, meaning red, and *kytos*, meaning cell), the white cells (leucocytes, from Greek *leukos*, meaning white), and the blood platelets.

When blood is shed it ceases, after a few minutes, being liquid and becomes solid, or, as we usually say, it clots (coagulates); after a few hours a straw-colored liquid begins to separate from the part which remains clotted, this liquid part of the clotted part is called the blood serum; so that the liquid part of living (i. e., unshed) blood is plasma, while the liquid part of the clotted blood is blood serum.

One of the most interesting things in immunity is the fact that if the blood should be shed and not be permitted to clot (e. g., by receiving into a solution of such chemical substances as potassium oxalate or citrate), so that the plasma should be obtained, no trace of any immune bodies can be found; yet allow this blood to clot and obtain the blood serum, we shall find all the immune bodies that the organism contains.

Just how this is brought about we do not know, but the fact remains that in studying the immune bodies we always speak of them as occurring in the blood serum although in the living body there is no such thing as blood serum (the latter existing only in the blood outside of the body).

Now after this short digression, let us return to the consideration of the immune bodies or antibodies.

The first statement to make in this connection is that the normal blood has the power to destroy bacteria; this power varies in different individuals; if the blood serum is kept for some time it will be noticed that this power rapidly diminishes. The substance responsible for this effect of blood is called complement.

We now know that there are two chief kinds of bacterial poisons—the exotoxins and the endotoxins, the former being the separable poisons, secreted by bacteria and circulating in the blood throughout the entire body, while the bacteria themselves do not enter the blood circulation and usually remain where they have become lodged; the latter are inseparable poisons and only are liberated from the bacterial body after the bacteria have died, the bacteria themselves having circulated in the blood (the third kind of bacterial poisons, namely, the bacterial proteins are unimportant since they are not specific). It is evident that since the poisons liberated by the bacteria are different, the antibodies must be different, and such is the fact.

Let us first consider the case of the exotoxin-producing bacteria, such as the diphtheria or tetanus bacilli; when these have lodged in the body, within a short time there will be produced an enormous amount of exotoxin; if the body should produce such substances that would kill the bacteria, this would be of very little use, because while it would prevent further elaboration of

exotoxin, nevertheless, there has already accumulated enough poison to kill the body; for this reason a substance is produced that does not attack the bacteria but neutralizes the exotoxin; this substance is called "antitoxin."

It is very important to know that the early opinion that the antitoxin actually destroys the exotoxin is erroneous; it prevents the action of exotoxin not by destroying it, but by neutralizing it, the mixture of exotoxin and antitoxin being neutral much in the same manner as the mixture of an acid or alkali is.

When bacteria act by producing not the exotoxin but the endotoxin, it is apparent that it would be useless for the body to produce antitoxins, and the body in such cases produces several antibodies (not one, as in the case of exotoxins).

1. **Bacteriolysins** (bacteria, and Greek *lysis*, meaning solution); these are the substances which cause the bacteria to swell up, become granular, and finally undergo a complete solution.

2. **Agglutinins** (from Latin, meaning glue); these are substances which cause the bacteria to lose their motility if they are motile, and then clump in small or large groups.

Agglutinins are not really immune bodies, because clumped bacteria are just as dangerous and virulent as those that are not agglutinated, and are mentioned here because of their importance in diagnosis of certain infectious diseases.

3. **Precipitins** are substances which cause bacterial culture filtrates when mixed with the blood serum to form precipitates. Like agglutinins they are not true immune bodies, but are formed by bacterial invasion, and for this reason are mentioned here.

4. **Opsonins** (from Greek *opsonein*, meaning prepare

food for) are substances which so act on bacteria as to make them more easily destroyed by the white blood cells (leucocytes), as will be mentioned later.

It is very important to remember that antitoxins are formed by injection of not only bacteria and their products, but of many other poisons of plant and animal origin.

Likewise lysins, agglutinins and precipitins may be produced by injection of numerous different substances; one of the most common examples is the injection of one animal with the red blood cells of another, whereupon the former will produce antibodies (lysins) against the red blood cells of the latter; such antibodies instead of being called bacteriolysins are called hemolysins ("blood dissolvers").

V. Theories of Immunity

There are two distinct explanations offered to account for the various phenomena of immunity, one of Metchnikoff, called "cellular theory" (because he ascribes the most important part to the action of various cells of the body) or "theory of phagocytosis" (from Greek *phagein*, meaning to eat and *kytos* meaning a cell); and the other of Ehrlich, called "humoral theory" because the greatest importance is ascribed to body fluids (humoral is from Latin *humor*, meaning fluid).

1. **Metchnikoff's Cellular Theory, or Phagocytosis.**— This theory is based on Metchnikoff's earlier observations that ameba (a unicellular organism) when about to receive its nourishment would flow about the food particle, surround and engulf it, and then ingest it, and under the microscope the gradual disappearance of the food particle could be observed.

Metchnikoff next observed that, when anthrax bacilli

were injected into frogs, leucocytes (which possess ameboid movement) would engulf and destroy the bacilli. This line of observations, continued in the animals and human beings, soon left no room for doubt that a similar process frequently took place; in fact, the actual engulfment and disintegration of gonococci, meningococci, staphylococci, and pneumococci is easily demonstrable.

Metchnikoff divides the phagocytic cells into two groups: 1. Macrophages ("big eaters") which are large mononuclear leucocytes and certain fixed tissue cells, such as those lining serous cavities (pericardium,* peritoneum,** and pleura,†) cells of the spleen, lymph glands, etc. This takes place especially in chronic infectious diseases, such as tuberculosis, syphilis, etc.

2. Microphages ("small eaters") are chiefly polymorphonuclear leucocytes,‡ they are especially concerned in acute infectious diseases, such as meningitis, etc.

Among the substances mentioned under the antibodies formed against the endotoxin-producing bacteria were the so-called opsonins; it is now well to emphasize their importance in connection with phagocytosis; the reason that not all bacteria are destroyed by phagocytosis is twofold: in some diseases, such as pneumonia, meningitis, etc., the number of leucocytes is increased from the normal 7,500 to 15,000, 20,000, and upwards per cubic millimeter; this is called leucocytosis; in some diseases there is a diminution of leucocytes, and this is called leucopenia; thus, first of

*Pericardium is the sac in which the heart is found.
**Peritoneum is the lining of the abdominal cavity.
†Pleura is the covering of the lungs.
‡Leucocytes (the white blood cells) are of five kinds:
1. Small mononuclear leucocytes (contain one nucleus),
2. Large mononuclear leucocytes (contain one nucleus),
3. Polymorphonuclear leucocytes (contain one nucleus),
4. Basophile leucocytes (granules stain blue),
5. Eosinophile leucocytes (granules stain red).

all, for a successful phagocytosis there must be a leucocytosis; but even that alone is insufficient, as bacteria may repel the leucocytes; this is called negative chemotaxis (chemical attraction); this is the reason opsonins are so important because they help attract the leucocytes (i. e., exert positive chemotaxis) and render bacteria more easily digestible by leucocytes.

2. **Ehrlich's Humoral Theory.**—According to Ehrlich every body cell consists of a central part which is concerned in its function (that is, if it is a muscle cell its function is contractile, if it is a gland cell its function is to secrete, etc.), and another part which is concerned in receiving food particles; for the latter purpose it is conceived that each cell possesses chemical affinities or as Ehrlich calls them "side chains," which are the means of attracting to the cell the various nutritive substances.

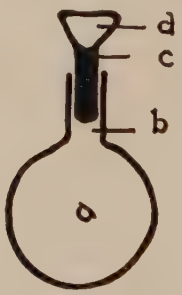


Fig. 9.—Ehrlich's theory of immunity. *a*, body cell; *b*, the receptor, or immune body; *c* and *d* bacterial poison; *c*, haptophore (anchoring) group; *d*, toxophore (poisonous) group.

The poisonous material, such as products of bacteria, may also become attached to the body cell through its side chains, and, in this manner, injure the cell (Fig. 9). If the poison is severe enough the body cell so injured will die; if not, it will only be damaged; since the body

cell must have food, and since its side chains are not useful any more (having combined with the bacterial poisons), these side chains with the bacterial poisons attached to them, will be cast off into the blood stream, and new side chains will be formed (Fig. 10). But when new side chains are produced, not only enough are formed to replace the old ones, but many more than are really necessary, according to Weigert's law of overproduction or overcompensation—if, for example, only six side chains have combined with bacterial poison, and, having become useless to the body cell, were cast off, then, when regeneration of side

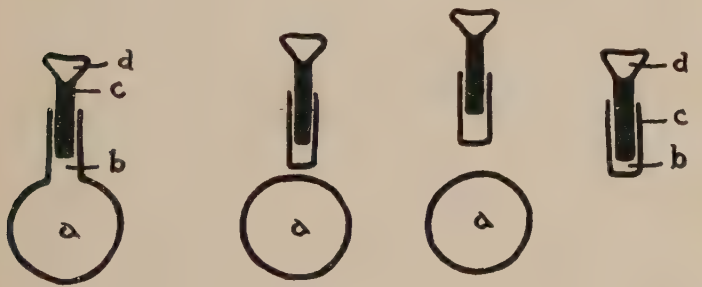


Fig. 10.—Side-chains, with bacterial poison attached, cast off free into circulation. *a*, body cell; *b*, receptor or immune body (side-chain); *c* and *d*, bacterial poison.

chains takes place, several hundred will be formed instead of just six. The body cell does not need them; what becomes of them? Not being needed, they are cast off into the circulation and there combine with the poisons which originally were the cause of their formation (Fig. 10). In order to understand the importance of this, let us take a concrete example: let us suppose that a body cell was injured by diphtheria exotoxin; let us further suppose that four side chains combined with this exotoxin; the body cell is injured but re-

covers; these four side chains combined with the exotoxin are cast off; new side chains are formed—not four but many more, according to Weigert's law; four of these new side chains are retained by the body cell to replace the old ones, the rest are thrown off into the circulation; having been formed as a result of injury to the cell by diphtheria exotoxin, these free side

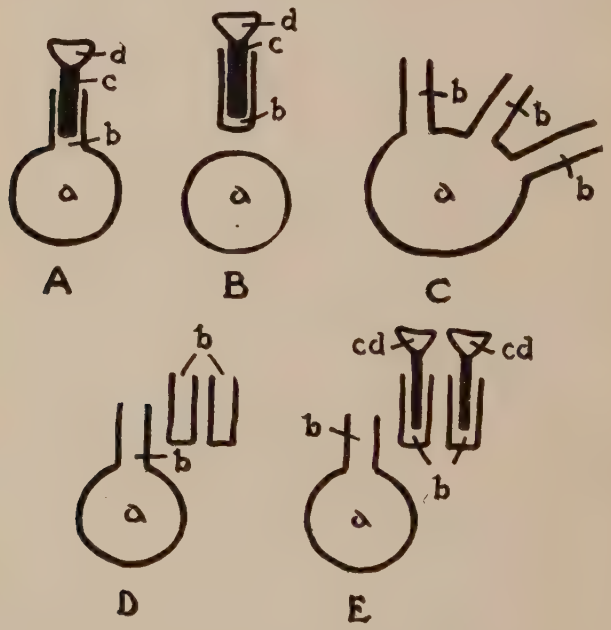


Fig. 11.—Illustration of Ehrlich's theory in case of diphtheria.

A. A body cell (*a*) is injured by diphtheria toxin; (*cd*) through the union of the latter with the receptor (immune body on side chain, *b*).

B. As a result of this the toxin and the receptor (immune body or side chain) are thrown off.

C. New receptors are formed to replace the one which had been thrown off, but, according to Weigert's law of overproduction or overcompensation, instead of one receptor many more (three in the illustration) are produced.

D. Only one receptor is needed to replace the lost ones, and the other two receptors are cast off.

E. These two free receptors (immune bodies or side chains) having been produced as a result of stimulation by diphtheria toxin, have now a special affinity for it and will, whenever they meet it unite with it, thus protecting the body cell by intercepting the toxin (*cd*).

chains, circulating in the blood, have the same affinity for the diphtheria exotoxin as if they were attached to the body cell, and because of this affinity, wherever they will meet the diphtheria exotoxin, they will combine with it, and, in this way, protect the body cell; because if it were not for their combining with the exotoxin, the latter would reach the body cell and would there combine with it by means of the side chains which remained there (to replace the four original side chains), and would do the same damage to the cell as it had done before (Fig. 11).

It is quite clear, therefore, that these free side chains are the antitoxins—they neutralize or combine with the exotoxins. This explains how a patient recovers from diphtheria; it explains why he remains immune (for some time or forever) after one attack of the disease; and, finally, it explains why such blood if injected into another animal would protect the latter from such infection. After the horse has been several times injected with the diphtheria exotoxin, his blood is full of these antitoxins (the side chains) and when injected into the patient, these side chains combine with the exotoxins in the patient's blood and thus protect him, in not allowing the exotoxins to reach the body cells and injure them. If a certain individual has no corresponding side chains for a certain exotoxin, in the first place, he will never be infected with this kind of exotoxin—natural or inherited immunity.

From his observations Ehrlich concluded that the toxin molecule consists of two parts: the haptophore group (the "carrying" part) which unites with the side chain of the body cell, and the toxophore group (the "poisoning" part), which does the actual damage (Fig. 12).

Some of the poisonous substances are attached di-

rectly to the side chain, as we have seen in the example of the diptheria exotoxin, such side chains, as for example, antitoxins, are called receptors of the first order.

Such side chains as agglutinins and precipitins are called receptors of the second order and differ from the antitoxins in that they have not only the combining group but also a digesting group (because the agglutinins and the precipitins not only unite with bacterial substances, as the antitoxins do with exotoxins,

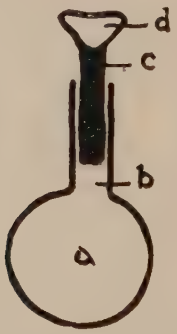


Fig. 12.—Ehrlich's conception of a toxin molecule. The toxin molecule (*cd*) consists of two parts: *c*, which combines with the receptor or side-chain *b* and is called the haptophore (the carrying or anchoring) group, and *d*, which contains the poisonous part and is called the toxophore group.

but also cause clumping and precipitating; hence the need of the digesting group). (Fig. 13).

Of the greatest interest and importance are the side chains, such as lysins (bacteriolysins, hemolysins, etc.), which are called receptors of the third order; we know experimentally that when an animal is injected with endotoxin-producing bacteria, e. g., cholera spirilla, its blood serum contains bacteriolysins; that means that if this animal's blood serum is mixed with cholera spirilla the latter will be destroyed—just what happens in the animal's blood; now, if this animal's blood serum

should be heated to 56° C. for thirty minutes, and then be mixed with cholera spirilla no such bacterial destruction will take place; this would seem to indicate that the bacteriolysins must have been destroyed by heat; yet, if to this heated blood serum a little normal blood serum from another animal is added, prompt destruction of bacteria will take place; this means that bacteriolysis is produced by the joint action of two substances: the specific immune body, produced by the animal

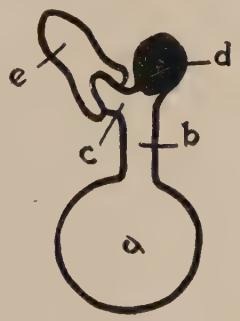


Fig. 13.—The receptor of second order (agglutinins and precipitins). *a*, body cell; *b*, receptor (immune body or side-chain), consisting of two parts; *c*, anchoring group, *d*, digesting group; *e*, bacterial poison.

as a result of injection (the antibody or the bacteriolysin) which is not destroyed by heating the blood serum up to 56° C. for thirty minutes, and a nonspecific substance, present in all normal as well as immune blood serum, and which is destroyed by heating up to 56° C.; this normal substance is the same substance which has been mentioned before in the section on immunity and to which a slight normal bactericidal (bacteria-killing) property of the blood is due; it is called complement.

We thus see that the receptors of the third order, such as bacteriolysins or hemolysins (which destroy the

red blood cells), do not act upon bacteria or red blood cells, as the case may be, alone, but in conjunction with the complement; because of this the side chain receptor of the third order has two "chains," one by which it combines with the bacterium or red blood cell or whatever had been injected into the animal, and the other for combining with the complement. (Fig. 14.)

While Ehrlich's humoral theory has very satisfactorily explained the production of all varieties of antibodies, for both the exotoxin and the endotoxin pro-

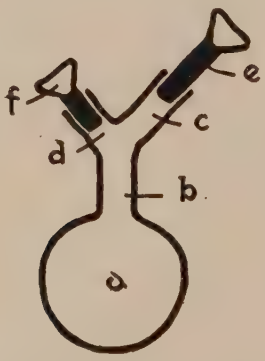


Fig. 14.—The receptor of third order (bacteriolysis, hemolysin). The receptor (side-chain or immune body), *b* consists of two parts; *c*, which unites with bacterium *e* and *d* which unites with complement (*f*).

ducing bacteria, it fails to take into consideration the phagocytosis of Metchnikoff—a phenomenon which doubtless plays a tremendously important part in many infections.

On the other hand, Metchnikoff's theory of phagocytosis does not explain as many of the phenomena of immunity, as does Ehrlich's, and it is necessary to use both theories in order to have a clear picture of immunity.

VI. Anaphylaxis

Anaphylaxis is the opposite of prophylaxis—the latter is the prevention of infection, while anaphylaxis (from Greek *ana,* meaning against or no, and *phylaxis,* meaning protection) means an abnormal sensitiveness to infection.

By anaphylaxis we mean sensitiveness due, not only to bacteria, but to any other proteins; it explains why some people are left more susceptible to infectious disease after one attack than ever before; why some people can not eat certain kinds of food, as e. g., shell fish; why in some people injection of a serum (not only immune serum such as diphtheria, but even a normal horse serum which is given to control hemorrhage) produces dangerous symptoms of shock, and, at times, even death.

The main facts about anaphylaxis can be summed up as follows:

1. Any protein—whether bacterial plant or animal—can produce anaphylaxis.

2. The second dose of such protein may be infinitesimally small.

3. If the animals do not develop anaphylaxis after the second injection, they remain forever immune.

4. The greatest liability for the development of anaphylaxis is between the tenth and fifteenth day after the first injection.

5. Anaphylaxis can be prevented by giving the second injection before the tenth day.

6. Anaphylaxis can be passively transferred from mother to her offspring, that is, if mother should be given one injection, and her offspring should be injected with her blood, and then with an injection of the sensitizing substance, the offspring will develop anaphylaxis.

No definite theory is as yet acceptable which can account for all the phenomena of anaphylaxis.

Practically, the knowledge of anaphylaxis is very important.

1. Some diseases can be diagnosed by taking advantage of the fact that if a person is unusually sensitive to a certain infection because he is infected with it, an injection of a specific bacterial protein will cause a sharp reaction and will help diagnose his infection; on this principle are based such tests as tuberculin; luetin (for syphilis), and mallein (for glanders), tests; a small amount of specific bacterial protein is injected into the skin, and if a person suffers from an infection caused by this particular bacterium, a marked reaction shows itself both at the site of injection and in the general condition of the patient.

2. It explains the mysteries of the so-called "serum-disease"—disease which follows the administration of various serums. In many cases the serum should be given in very small doses before the full amount given in order to avoid the possibility of anaphylaxis.

CHAPTER V

THE STUDY OF BACTERIA AND GENERAL BACTERIOLOGIC TECHNIC

I. Study of Bacteria in Living State

The study of bacteria in living state is carried out in what is called the "hanging drop" preparation: A "hollow" slide is used—an ordinary glass slide, usually 3 inches by 1 inch, in the center of which a circular depression is made; the edges of it are smeared with vaseline, then a drop of fluid medium in which the bacteria have been grown is placed in the center of an ordinary cover-glass (this is a piece of very thin glass, about 1 inch square); the majority of books then suggest to lift up the cover-slip and then invert it over the concavity of the "hollow" slide, so that the drop hangs over the depression of the slide; my custom is to do just the opposite; namely, without disturbing the cover-glass, I lift up the slide and invert it over the cover-glass so that the vaseline around the depression of the slide just touches the cover-slip, and then put the slide down on the table with the cover-glass uppermost; in this way the danger of breaking the very thin cover-glass in handling it with fingers is eliminated (the danger of crushing it when inverting the glass slide over the cover-glass does not exist since the vaseline over the depression of the slide acts as a buffer between it and the cover-glass); besides, another source of danger is done away with; namely, that of spilling or smearing the bacterial drop while in-

verting it over the glass slide—a very real danger, too, when handling virulent bacteria.

If the bacteria have been grown on a solid medium, some growth should be taken from the culture tube with a platinum needle and emulsified with physiologic salt solution (0.8 per cent solution of NaCl) and a drop of this emulsion should then be transferred to the cover

Fig. 15.—*A*, staining support with a gas burner underneath; *B*, staining bottles; *C*, Petri dishes.

slip. The preparation is then ready for the microscopic examination.

The main object to be accomplished by studying the bacteria in this way is to determine whether or not they are motile.

II. Study of Bacteria in Stained Preparation

For this purpose a drop of fluid culture or a drop of bacterial growth emulsified in a little physiologic salt

solution, is spread thinly on a glass slide or a cover-slip, is allowed to dry in the air, is then rapidly passed several times through the flame of a Bunsen burner (this process fixes the preparation), and is now ready for staining. (Fig. 15.)

Ordinary Stains (Gruebler's or Merck's)

The stains used for bacteriologic purposes are anilin dyes, which are used as saturated solutions, either alcoholic or aqueous (in water).

The most commonly used stains are:

Methylene blue: aqueous saturated solution is 6.7 per cent.
Methylene blue: alcoholic saturated solution is 7 per cent.
Gentian violet: aqueous saturated solution is 1.5 per cent.
Gentian violet: alcoholic saturated solution is 4.8 per cent.
Fuchsin: aqueous saturated solution is 1.5 per cent.
Fuchsin: alcoholic saturated solution is 3 per cent.

The alcohol used here is understood to be 95 or 96 per cent alcohol.

After the bacterial preparation has been fixed, any of the above-mentioned stains is poured on the slide, and is allowed to remain there for two to five minutes; most frequently methylene blue is used for routine examinations. The stain is then poured off the slide, the preparation is washed in water, dried on filter paper, a small drop of cedar oil is placed on the cover-glass or slide, or, if the preparation is to be preserved, a drop of Canada balsam is to be put between the glass slide and the cover-glass, and a drop of cedar oil is then placed on the cover-glass; the preparation is then ready for microscopic examination. (Fig. 16.)

No directions for the use of the microscope are given,

for it is my conviction that no directions will ever do any good; the pupil must be individually taught just how to handle the microscope and care for it.

Special Staining Methods

A. **Gram's Method.**—This is an extremely important method which permits differentiation of bacteria into two classes: the so-called Gram-positive and Gram-negative bacteria; this will be made clear immediately after the

Fig. 16.—Microscope (*A*) and artificial illumination (*B*).

description of the method of staining which is as follows:

1. Prepare the bacterial smear in the usual manner on a glass slide or a cover-glass, as described in the section on the Study of Bacteria in Living State.

2. Cover the preparation for five minutes with the anilin gentian violet stain, which is prepared as follows: 5 c.c. of anilin is shaken thoroughly with 125 c.c. of dis-

tilled water and the mixture is filtered through moist filter paper.

To 108 c. c. of the mixture add 12 c. c. of the saturated alcoholic solution of gentian violet.

3. Pour off the stain and cover it for thirty seconds with Gram's iodine solution, which is prepared as follows:

Iodine	1 gram
Potassium iodide	2 grams
Distilled water	300 c.c.

4. Pour off the iodine solution and decolorize with absolute alcohol until it ceases to discharge the blue color.

5. Wash the preparation in water, and leave some water on the slide.

6. Pour a few drops of carbol-fuchsin stain and immediately wash the preparation in water.

7. Dry on a filter paper and put a drop of cedar oil.

The carbol-fuchsin stain is prepared as follows: Dissolve 1 gram of basic fuchsin in 10 c. c. of absolute alcohol, and mix the 10 c.c. of this alcoholic solution of fuchsin with 90 c. c. of 5 per cent aqueous solution of carbolic acid.

When stained by this method, it will be found that some bacteria have retained the gentian violet stain, while others have lost it and have taken up the carbol-fuchsin stain. Those bacteria which, when stained by this method, have retained the gentian violet (i. e., are stained violet) are called Gram-positive, while those which have lost it and have taken up the carbol-fuchsin (i. e., are stained red) are called Gram-negative. The following table shows the most important bacteria as belonging to either one or the other class:

BACTERIAL CLASSIFICATION ACCORDING TO GRAM'S STAIN

GRAM—POSITIVE (Retain the gentian violet)	GRAM-NEGATIVE (Lose gentian violet, take carbol fuchsin and appear red)
Staphylococci	Bacillus typhosus
Streptococci	Bacillus coli
Pneumococci	Meningococcus
Bacillus of anthrax	Gonococcus
Bacillus diphtheria	Glanders bacillus
Bacillus of tetanus	Bacillus pyocyaneus
Bacillus of tuberculosis	Bacillus of influenza
Bacillus aerogenes Capsulatus ("Gas" bacillus)	Plague bacillus
	Cholera spirillum
	Friedländer's bacillus

B. **Stain for Spores.—**

1. Make and fix the preparation in usual manner.

2. Cover with Loeffler's alkaline methylene blue and heat the stain until it boils, remove it from flame, then heat again; repeat this for one minute (Loeffler's alkaline methylene blue is prepared by mixing 30 c.c. of saturated alcoholic solution of methylene blue with 100 c.c. of 1:10,000 solution of potassium hydroxide in water).

3. Rinse in water.

4. Decolorize with a mixture of 98 c.c. of 80 per cent alcohol and 2 c.c. of nitric acid, until all blue has disappeared.

5. Rinse in water.

6. Dip three to five seconds in a mixture of 10 c. c. of saturated alcoholic solution of eosin and 90 c.c. of water.

7. Rinse in water and blot.

Spores are stained blue and the body of bacteria are stained pink. (See Fig. 4.)

C. **Stain for Capsule.—**

1. Smear the bacteria in a drop of beef blood serum, dry and fix by heat in the usual manner.

2. Stain for a few seconds with a mixture of 5 c.c. of saturated solution of gentian violet and 95 c.c. of distilled water. Hold the preparation flooded in this over a flame until it steams.

4. Wash off the stain with 20 per cent aqueous solution of copper sulphate.

5. Blot (do not wash in water).

The capsule appears as a blue halo around a dark purple cell body. (See Fig. 2.)

D. **Stain for Flagella.**—(See Fig. 3.)

Use bacterial emulsion from young cultures grown on agar media (see section on Culture Media).

1. Dry the preparation in air and fix by heat.

2. Pour the following mixture, freshly filtered: 10 c.c. of 20 per cent aqueous solution of tannic acid, 5 c. c. of saturated aqueous solution of ferrous sulphate, and 1 c. c. of saturated alcohol solution of fuchsin.

Allow this mixture to remain thirty to forty-five seconds, heating it gently.

3. Wash thoroughly in water.

4. Stain for one to two minutes, warming gently, with the following freshly filtered mixture:

50 c.c. of 5 per cent anilin gentian violet.
0.05 gram of sodium hydroxide.

5. Wash in water and blot.

E. **Stain for Tuberculosis and Other "Acid-Fast"**

The Ziehl-Neelsen Method.

1. Make the preparation (from the culture, urine, sputum, etc.) in the usual manner, dry in air and fix with heat.

2. Pour the carbol-fuchsin stain (its preparation has been given in the section on Gram's Method), and heat gently until it steams; continue this for three to five minutes, this had best be done on a special staining support under which there is a gas pipe with numerous perforations, connected by a piece of rubber tubing with the source of gas supply; this method permits of regulating the amount of heat just sufficient for steaming, thus preventing the boiling. (See Fig. 15.)

3. Decolorize with 1 per cent hydrochloric acid or 5 per cent nitric acid in alcohol, then with 90 per cent alcohol until no pink color is discharged.

4. Wash in water.

5. Stain in aqueous saturated solution of methylene blue for one to two minutes.

6. Rinse in water, and blot.

By this method the tubercle bacilli are stained red, while everything else (including other bacteria) is stained blue.

The principle of this stain is as follows: the tubercle bacilli do not stain with ordinary dyes—for this reason, hot carbol-fuchsin is used; but other bacteria which may be present (in the sputum, urine, etc.) are also stained (red). When strong decolorizing acid is applied, however, all other bacteria lose the carbol-fuchsin stain, and take the methylene blue, while the tubercle bacillus does not give up the carbol fuchsin in spite of the action of the strong acid solutions (for this reason it is called an "acid-fast" organism), and remains red. Other acid-fast bacteria are the smegma and the leprosy bacilli.

F. **Stain for Malaria and Blood Films.**—The best one is Wright's stain; which is prepared as follows (avoid using the commercial stain, as the various specimens vary greatly in strength, and each one has to be tried out

before it can be safely used): 200 c.c. of 1 per cent solution of methylene blue in 0.5 per cent solution of sodium bicarbonate in distilled water are steamed in Arnold's sterilizer for one hour.

Upon cooling, 1,000 c.c. of 0.1 per cent aqueous solution of eosin (water soluble) is added, until a metallic scum appears on the surface of the mixture. The precipitate which has been formed is collected by filtration, dried, and a satured solution is made in methyl (wood) alcohol. This is filtered and diluted with one-fourth its bulk of methyl alcohol.

To look for malarial organisms, make a preparation in the same manner as for the study of blood, the so-called preparation for a differential count: clean the patient's ear or the tip of a finger with alcohol, dry and prick with a needle or a blood lancet; wipe away the first drop of blood, collect the next drop on a scrupulously clean glass slide; apply another slide, held at angle of about 45° so that the edge of the second slide just touches the first slide where the drop of blood is; this will cause the latter to spread, by capillary attraction, along the edge of the second slide; drag it along the first slide, holding it firmly against it, and you will get a thin, even "blood film." This is allowed to dry, and the slide is then covered with the stain for one to two minutes (this does not stain but merely fixes the preparation because of the methyl alcohol present in the stain). Now add a few drops of distilled water to the film covered with stain until a distinct metallic luster appears on the surface. Leave this on for five to ten minutes. Wash in distilled water and dry.

In such a preparation the red blood cells appear pink (if they contain the malarial parasites the latter are seen as being in—not on—the red blood cells and have the

various appearances as described in the section on Malaria); the leucocytes appear as follows: the polymorphonuclears are body pink and the nuclei (from one to five segments) purple, the small and the large mononuclears (the lymphocytes) show a narrow rim of pink protoplasm and large purple nucleus, the mast cells show purple granules, and the eosinophiles show red granules.

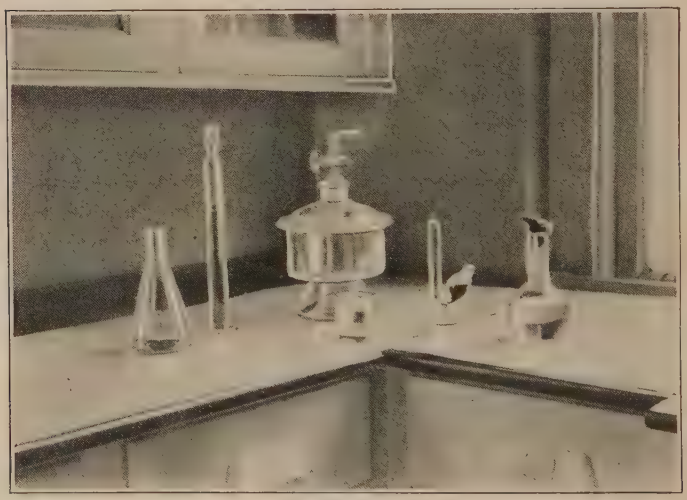


Fig. 17.—Miscellaneous glassware. *A*, Erlenmeyer flask; *B*, graduate glass cylinder; *C*, anaerobic apparatus; *D*, fermentation tube; *E*, boiling flask.

III. Plating and Anaerobic Cultures

See Section on Applied Bacteriology.

IV. Culture Media

This is one of the most important things in bacteriology, not only because we can not study bacteria unless we grow them, but because the different ways in

which the different bacteria grow on media and the different changes which they produce in the culture media (fermentation of sugars, the production of acid, coagulation of milk, liquefaction of gelatin, etc.) constitute one of the most valuable means of bacterial differentiation and identification. (Fig. 18.)

By a culture medium (plural: culture media) we mean any substance on which bacteria grow outside the animal body.

Before the various culture media can be prepared, several other things have to be attended to.

Preparation of Glassware.—If the glassware is new, immerse it in 1 per cent solution of hydrochloric acid, then wash it in 1 per cent sodium hydroxide, and, finally, wash it in running water. Old glassware containing infectious material should first be autoclaved for one hour, then emptied, and boiled for one hour in soap suds, then cleaned with a brush, and sterilized for one hour in the hot-air sterilizer at 150° C. If it is very dirty, it may, after it has been autoclaved, be immersed for twelve to twenty-four hours in a mixture of three parts of saturated aqueous solution of potassium bichromate and one part of sulphuric acid.

The usual glassware for culture media consists of test tubes. Erlenmeyer and Florence flasks and Petri dishes, which are shown in accompanying illustrations, should all be plugged with nonabsorbent cotton; the best and easiest way to plug the test tubes, is to take a piece of cotton, two inches square, put over the mouth of the test tube and push it in for a distance of one inch with a pencil or a glass rod.

The Composition of Culture Media.—Most culture media contain meat, agar, peptone, salt, and water. With these substances as a basis, many so-called "enrich-

ing'' substances may be added, in order to enable us to grow the bacteria which require especially rich foodstuffs; of such fastidious bacteria we may mention pneumococci, streptococci, gonococci, influenza bacilli, etc. Of the ''enriching'' substances those most frequently used are various sugars, blood (either whole or ''whipped'' —defibrinated in order to prevent the coagulation), ascitic fluid (the fluid removed from the patients suffering from dropsy), etc.

Fig. 18.—Making a transfer of a culture.

Speaking generally, the following requirements concerning the general nature of the ingredients used in preparing the culture media should be borne in mind:

1. Distilled water should be used unless tap water is specified.

2. All chemicals used should be of the highest grade obtainable (the so-called ''chemically pure,'' C. P.)

3. The peptone should be Witte's peptone.

4. The meat should be lean, fresh beef.

Titration of Media.—Titration of media is a very important step. By titration of media is understood the adjustment of the reaction of the media, that is, whether they are acid or alkaline; the bacteria are, as a rule, very sensitive to this, and for this reason titration should be most carefully carried out in all cases.

The color indicator used is 0.5 per cent solution of phenolphthalein in 50 per cent alcohol.*

To determine whether the medium is acid or alkaline, take a few c.c. of the medium and drop one or two drops of the phenolphthalein solution; if the medium is acid, no color develops; if alkaline—a pink color appears.

Most of the media are acid because of the presence of meat acids.

For the actual titration of acid medium the so-called "one-twentieth normal solution of sodium hydroxide" is used, which is expressed as follows: N/20 NaOH, while for the titration of the alkaline medium "one-twentieth of normal solution of hydrochloric acid" is used, which is expressed as follows: N/20 HCl. By a "normal" chemical solution we understand "the molecular weight of a substance, expressed in grams, dissolved in a liter (1,000 c.c.) of distilled water; in the case of sodium hydroxide the formula is NaOH, and its molecular weight is that of sodium (Na) which is 23 plus that of oxygen (O) which is 16 plus that of hydrogen (H) which is one; that is, $23+16+1=40$; the molecular weight of sodium hydroxide is, therefore, 40; now, if we dissolve 40 grams of it in 1,000 c. c. of distilled water, we will have a "normal" solution of sodium hydroxide; if we wish to make one-twentieth normal solution take 1/20 of

*Any per cent solution of alcohol can be prepared from the standard 95 per cent alcohol by taking as many cubic centimeters of 95 per cent alcohol as the percentage desired and filling it with water up to 95 c. c.; in this case, for example, take 50 c. c. of 95 per cent alcohol and add 45 c. c. of water.

40, i. e., 2 grams, and dissolve it in 1,000 c. c. of distilled water; to make one-tenth normal solution take 1/10 of 40, i. e., 4 grams of NaOH in 1,000 c. c. of water, etc.

In the case of hydrochloric acid the formula is HCl; the molecular weight of hydrogen (H) is 1; that of chlorine (Cl) is 35.5; therefore, the molecular weight of HCl is 36.5; now HCl being a liquid, we measure it in cubic centimeters (c.c.) and not in grams; take 36.5 c.c. of hydrochloric acid and add distilled water up to 1,000 c.c.; to make one-twentieth normal solution of hydrochloric acid, take 1/20 of 36.5, i. e., 1.825 c.c. of hydrochloric acid and add distilled water up to 1,000 c.c.

Titration of Acid Medium.—Into an evaporating dish of 100 c.c. capacity pour 5 c.c. of the medium to be titrated, and add 45 c.c. of distilled water. Boil for three minutes over a free flame (to drive off the carbon dioxide). Add one c.c. of the phenolphthalein solution; the medium being acid, no color will be present, as explained above. Now from a special tall tube called a "burette" (this is a tall, narrow tube, usually of 50 c.c. capacity graduated in tenths of a c.c., and provided with a stopcock to permit removal of small quantities of fluids), a few drops of the "twentieth normal solution of sodium hydroxide" (N/20 NaOH) are permitted to drop into the evaporating dish containing 5 c.c. of the medium and 45 c.c. of distilled water; at first no color is present, but little by little a pink color will develop, which, however, will disappear upon stirring with a glass rod. When a permanent pink color—that is, one which will not disappear upon stirring—has been reached, we know that the neutral point (that is, neither acid nor alkaline) has been obtained.

Titration of Alkaline Media.—Titration of alkaline media is carried out in the same manner as that of the

acid medium, except that a N/20 HCl (one-twentieth normal solution of hydrochloric acid) is used. When 1 c.c. phenolphthalein solution is added, every drop of the hydrochloric acid solution added will cause a pink color, as explained above, but as more and more of the N/20 HCl is added the color will gradually fade away, and when the color has completely disappeared, the neutral point has been reached.

This is, in other words, just the opposite of the acid titration: there we work from no color to a permanent pink, and here from pink to no color.

The Calculation.—Let us suppose that in titrating an acid medium we had to use 2 c. c. of N/20 NaOH to make our medium in an evaporating dish (5 c.c. of medium, *not* 50 c.c., as 45 c.c. was distilled water). The calculation is then easy:

If 5 c.c. of the medium requires 2 c.c. of N/20 NaOH for neutralization, then 100 c.c. of the medium requires 20 times as much (because 100 c.c. is twenty times as great as 5 c. c.); that is, $2\times20=40$ c. c. of NaOH.

If we had prepared 1,000 c.c. of the medium (a liter), this 1,000 c.c. of the medium will require 10 times as much of N/20 NaOH as 100 c. c. did; i. e., $40\times10=400$ c.c. of N/20 NaOH. Thus, to our 1,000 c.c. of the medium we must add 400 c.c. to the N/20 NaOH to make it neutral; but adding 400 c. c. will make our medium too bulky; for this reason instead of adding one-twentieth of NaOH we will add the normal solution of NaOH; how much? Since the normal solution of sodium hydroxide is twenty times as strong as the normal twentieth solution, it is evident that we must use one-twentieth of the normal twentieth solution, i. e., instead of 400 c.c. of N/20 NaOH we will use 20 c.c. of N/1 NaOH.

The calculations of the alkaline titration do not differ in any way from the above.

For ordinary purposes we use a medium which is not neutral but is about 1 per cent; i. e., requires one c.c. of N/1 NaOH per 100 c.c. of the medium, or 10 c.c. per 1,000 c.c. (liter). In the above calculation, therefore, we should, instead of adding 20 c.c. of N/1 NaOH to the liter of medium, have added only 10 c.c. of it, thus leaving the medium still 10 c.c. of N/1 NaOH short for complete neutralization; that is, leaving it 1 per cent acid.

The acidity of the medium is expressed by the sign + and the alkalinity by that of —.

Tubing the Media.—After the media have been prepared they are poured into test tubes (about 10 c. c. into each) or into flasks (about 30 to 50 c.c. into each); this is usually done by pouring the medium into a funnel to the tip of which a rubber tubing had been fitted, with a pinchcock, and the tubes and flasks are so filled from this; a special apparatus has been devised (see Fig. 19) which consists of a large glass bulb with a glass outlet, a stopcock, and a gauge which permits to pour into the tubes or flasks equal amounts of the medium.

Sterilization of Media.—The ordinary media which do not contain sugar, glycerin, gelatin or animal serum, are sterilized in the steam pressure sterilizer (autoclave) at fifteen pounds pressure for fifteen to thirty minutes; the media containing substances which are apt to be injured by the high temperature must be sterilized by the fractional method (see the section on Destruction of Bacteria); that is, by exposure for twenty minutes in Arnold's sterilizer, on three successive days.

The media containing animal serum must be sterilized in water-bath or hot-air sterilizer at the temperature of 60° to 70° C. for half an hour on three successive days.

Some media are best sterilized by passing through the special bacterial filters (Berkefeld's or Chamberland's) which are made of unglazed porcelain or earthenware.

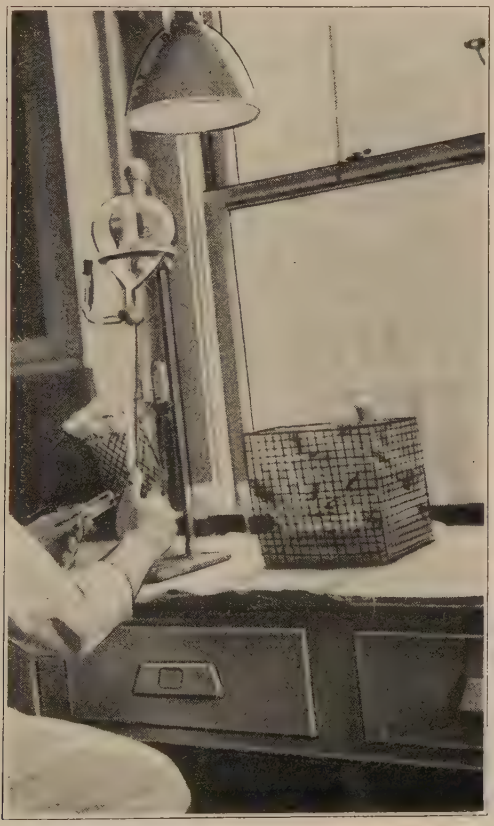


Fig. 19.—Tubing the media.

Slanting of Media.—The solid culture media, after they have been sterilized, usually are slanted, that is, the tubes are laid down on a desk or table so that the upper

end of the test tube rests on a ledge or glass tubing, in order that the medium, when coagulated, will form a slant in the tube (Fig. 16).

Actual Preparation of Various Culture Media

1. Meat Extract Agar

Agar is a Japanese seaweed.

Meat extract agar is the most commonly used medium. Staphylococci typhoid and colon bacilli grow well on this. Other bacteria require richer media.

(a) Pour into an agate vessel 1,000 c.c. of distilled water, add 15 grams of shredded (not powdered) agar, 10 grams of Witte's peptone, 5 grams of sodium chloride and 5 grams of Liebig's meat extract.

(b) Heat until agar is completely dissolved (thirty to forty-five minutes).

(c) Add enough water to make up for loss by evaporation.

(d) Cool to 60° C. and add whites of two eggs.

(e) Heat in Arnold's sterilizer for thirty minutes, stir and heat fifteen minutes.

(f) Add water to make up for loss by evaporation. Filter through cotton.

(g) Titrate to the desired reaction (usually about 0.2 per cent acid, to phenolphthalein).

(h) Tube and sterilize in autoclave for thirty minutes at 15 pounds pressure. Slant.

2. Meat Infusion Agar

Meat infusion agar is one of the richer media, suitable for culturing streptococci, etc.

(a) In a two-liter Erlenmeyer flask pour 1,000 c.c. of water and put 1 lb. of finely chopped lean beef, and put in the ice box over night.

(b) Boil for twenty to thirty minutes, make up loss of water, and filter through a cotton gauze filter (2 or 3 layers of gauze lined with cotton; when no more fluid runs through, gather the edges of the gauze over the cotton and squeeze it dry).

(c) Add 10 grams of peptone and 5 grams of sodium chloride and heat (not over 50° C.) until peptone is dissolved.

(d) Add 15 grams of shredded agar and boil until agar is dissolved. Make up to 1,000 c.c.

(e) Heat for thirty minutes in Arnold's sterilizer, stir, and reheat for fifteen minutes.

(f) Titrate, adjust the reaction to 0.2 to 0.5 per cent acid, filter through cotton and tube.

(g) Sterilize thirty minutes in Arnold's sterilizer on three successive days. Slant.

3. Meat Extract Broth

Meat extract broth is a liquid medium corresponding to meat extract agar.

(a) To 1,000 c.c. of water add 10 grams of peptone, 5 grams of Liebig's meat extract, and 5 grams of sodium chloride.

(b) Heat until dissolved, and add water up to 1,000 c.c.

(c) Titrate and filter through paper (if not clear, add whites of two eggs, heat for thirty minutes in Arnold's sterilizer, and then filter through cotton).

(d) Tube and sterile in autoclave thirty minutes at 15 pounds pressure.

4. Meat Infusion Broth

Meat infusion broth corresponds to meat infusion agar. It is prepared exactly as the meat infusion agar (Medium No. 2) except that the agar is omitted, and sterilized like it (in Arnold's sterilizer for thirty minutes on three successive days).

5. Sugar-free Broth

Sugar-free broth is used for making different sugar media. Prepare the broth just as the meat extract broth (No. 3) is prepared.

After the step *b*, add 10 c. c. of a twenty-four-hour-old culture of colon bacillus. Then incubate at 37° C. for twenty-four hours. (The colon bacillus will destroy all sugar present.) Heat for thirty minutes in autoclave at 15 pounds pressure. Add water up to 1,000 c.c., filter through paper and titrate.

6. Sugar Broths

After No. 5 has been prepared (the sugar-free broth) add different sugars (dextrose, lactose, saccharose, etc.), in proportion of 1 per cent tube in fermentation tubes, and heat for twenty minutes (not more, as it will spoil the sugars) in Arnold's sterilizer on three successive days. (Instead of fermentation tubes one can use the following: tube in ordinary test tubes; when tubed, place into each test tube a very small tube (2x¼ inch) inverting it, so that its mouth rests against the bottom of the larger tube. During the sterilization the medium will be forced up into the smaller tube, and if fermentation will take place upon inoculation, the empty space where gas is collected in the small tube will show it.

7. Enriched Media

All of the meat infusion media (No. 2 and No. 4) can be enriched, by adding glucose 1 to 2 per cent, or a few drops of ascitic fluid or of defibrinated or whole blood, or glycerin.

When ascitic fluid is added greatest aseptic precautions should be observed, as after its addition no further sterilization by heat is permissible since it would coagulate the albuminous part of the ascitic fluid. An ordinary meat infusion agar is made as above described, tubed and sterilized. When ascitic fluid is to be added, melt the agar tubes by heating in water bath, cool to 45° C., then (with all aseptic precaution) add to each tube a few drops of ascitic fluid, flaming the test tubes before and after the addition; incubate all tubes at 37° C. for twenty-four hours to see if they are sterile.

For defibrinated or whole blood, do just as for the ascitic fluid agar, except that a few drops of defibrinated or whole blood is added. To obtain defibrinated blood, bleed a rabbit from the carotid artery or jugular vein into a sterile flask with 10 to 20 glass beads, shake the blood thoroughly, add a few drops to each tube of melted and cooled agar, and incubate for twenty-four hours to test sterility.

If sugar is added (usually glucose 1 to 2 per cent) sterilize for twenty minutes instead of thirty minutes in Arnold's sterilizer on three successive days.

8. Gelatin Agar

Gelatin agar is used to determine the gelatin-liquefying properties of bacteria.

(a) To 1,000 c.c. of water add 5 grams of Liebig's meat extract, 5 grams of sodium chloride, 10 grams of pep-

tone and 100 grams of best French sheet gelatin ("Gold Seal" brand).

(b) Dissolve by heating, add water up to 1,000 c.c.

(c) Cool to 60° C., add whites of two eggs, stir and heat for thirty minutes in Arnold's sterilizer.

(d) Add water up to 1,000 c.c. filter through cotton. Sterilize in autoclave for twenty minutes at 15 pounds pressure.

(e) Do not slant this medium, as it is inoculated by "stabbing."

9. Potato Media

(a) Large potatoes are well washed and scrubbed with a nail brush. Peel and wash in running water.

(b) Cylindrical pieces are removed with a large apple corer, and each cylinder is cut diagonally into two wedges.

(c) Immerse the wedges overnight in 1 per cent solution of sodium carbonate to correct the normal acidity.

(d) Insert the wedges into test tubes (if desired they can be first soaked in 25 per cent glycerin—the glycerin potato medium).

(e) Sterilize for thirty minutes in Arnold's sterilizer on three successive days.

10. Litmus Milk

Litmus milk medium is used for determining the power of bacteria to curdle the milk and produce acid (as shown by fading of the blue color and the tube turning red).

(a) Fresh milk is heated in a flask in Arnold's sterilizer for fifteen minutes.

(b) Put flask in ice box to allow the cream to separate. The cream is then syphoned off.

(c) Neutralize the acidity if in excess of 1.5 per cent.

(d) Add a few drops of a 5 per cent aqueous solution of litmus.

(e) Sterilize in Arnold's sterilizer for thirty minutes on three successive days.

11. Loeffler's Serum Medium

Loeffler's serum medium is used for diphtheria bacillus.

(a) Beef blood is collected in sterilized jars, allowed to clot, the clot is loosened by a glass rod, and jars are kept on ice overnight.

(b) Clear serum is pipetted off to sterile flasks.

(c) To three parts of the serum one part of 1 per cent glucose meat infusion broth is added, and the whole is tubed.

(d) It is then sterilized in a special sterilizer called "inspissator" at a temperature of 70° C. for two hours on six successive days.

12. Endo's Medium

Endo's medium is used for the differentiation of the typhoid and colon bacilli.

1. Prepare ordinary meat extract agar, except that it should contain 25 to 30 grams of agar per 1,000 c.c. of media, except the usual 15 grams, and it should be 0.2 per cent acid. Do not tube it, but keep it in flasks. When Endo's medium is wanted, melt the agar, add to it 1.8 c. c. of filtered 10 per cent solution of basic fuchsin in 95 per cent alcohol and 25 c.c. of freshly prepared 10 per cent aqueous solution of sodium sulphite crystals. Then add 10 grams of lactose (milk sugar) in 50 c.c. of sterile water. Sterilize for fifteen minutes in Arnold's sterilizer on three successive days.

On such a medium the typhoid colonies are colorless, while those of colon bacilli are red.

13. Russell's Double Sugar Medium

Russell's double sugar medium is used for the differentiation of the typhoid, paratyphoid and colon bacilli.

(a) Prepare ordinary meat extract agar (25 grams of agar per 1,000 c. c. of medium, 0.8 per cent acid) and keep it in flasks. When needed, melt it.

(b) Add enough of sterile 5 per cent aqueous solution of litmus (usually about 30 c.c.) to give the medium a distinct violet color.

(c) Add 0.1 per cent glucose (grape sugar) and 1 per cent lactose (milk sugar) dissolved in 50 c.c. of water, tube and sterilize in Arnold's sterilizer for fifteen minutes on three successive days. Slant. When inoculations are made smear the surface and also stab.

On such a medium typhoid growth will show a partial red color formation and no gas bubbles. The paratyphoid A and B will show a partial red color formation and gas bubbles in the red area, while the colon growth will turn the entire tube red and gas bubbles all over.

14. The Double Sugar—Lead Acetate—China Blue Medium

This medium is the most complete differentiating medium, as it tells at a glance whether the growth is one of typhoid, paratyphoid A, paratyphoid B, colon or B. enteritidis.

(a) Prepare ordinary meat extract agar, 0.3 per cent acid, and flask it.

(b) Heat some 2 per cent neutral lead acetate in sterile distilled water, at 100° C. for one hour in water-bath. 5 c.c. of this is added to 100 c.c. of agar (which has been

melted and cooled to 60° C.). Tube in Wassermann test tubes (4x½ inch).

(b) Prepare 1 per cent solution of China blue in distilled water.

(c) 0.4 c. c. of normal sodium hydroxide is added to 10 c.c. of 1 per cent solution of China blue, and the mixture is heated in water-bath for ten minutes at 100° C. (the blue color now changes to brown).

(d) 1.2 c.c. of this mixture is added to 100 c.c. of agar.

(e) 0.1 per cent glucose and 1 per cent lactose are also added, and the mixture is heated for ten minutes in water-bath at 100° C. Cool to 60° C., and pour on the first layer (step A) the same amount as the latter.

The changes caused by the different bacteria on this medium are shown in the following table.

Bacillus	Gas	Bottom Layer	Top Layer
Typhoid	No	Black	Pale blue
Paratyphoid A	Yes	No change	Pale blue
Paratyphoid B	Yes	Black	Colorless
Colon	Yes	Black	Deep blue
Enteritidis	Yes	No change	Colorless

15. Cornstarch Medium

This little-known medium is the best medium for gonococcus cultivation, also for pneumococcus and other bacteria which grow with difficulty. On this medium the gonococcus, which dies on all other media in four to five days, will live over four weeks, so that where cultivation and frequent transfers are to be made this medium will be found of greatest help.

(a) 500 grams of lean beef are added to 1,500 c.c. of water, and put in a flask on ice overnight.

(b) Strain through gauze, heat slowly to boil, add 15 to 17 grams of shredded agar; when dissolved, neutralize to 0.4 per cent acid (phenolphthalein).

(c) Cool to 60° C. add three whole eggs (beaten with a little water), put back on fire, heat quickly to boil, then turn the flame down to a simmer for fifteen minutes.

(d) Filter through cotton and gauze (both previously sterilized), add 10 grams of Squibb's cornstarch per 1,000 c.c. of medium, rub the starch with 60 c.c. of filtrate to a milky consistency, pour back into the rest of filtrate and shake well. (If lumps are present filter again and add more starch.)

(e) Sterilize in Arnold's sterilizer, shaking every ten minutes.

(f) Tube or flask. Autoclave fifteen minutes at 8 to 10 pounds pressure.

(g) Cool down to 42° C., tilt a few times (without wetting the stopper), slant overnight, then stand them up for a day. Good tubes show about $\frac{3}{8}$ of an inch of water of condensation.

16. Bile Medium. Used in Blood Cultures

Take 900 c.c. of fresh ox bile, 100 c.c. of glycerin and 20 grams of peptone. Put in small Erlenmeyer flasks (about 40 to 50 c.c. in each) and sterilize in Arnold's sterilizer for thirty minutes for three successive days.

17. Media for Tubercle Bacillus

The best media are those of Petroff; their preparation requires a good deal of skill, and the reader should consult Petroff's own description observing the most minute details.

A very good medium is the glycerin potato. Meat infusion agar with about 10 to 15 per cent of whole blood (not defibrinated) is also excellent.

One of the best media is that of Dorset:

(a) Carefully break 16 eggs into a flask, shake it until the whites and yolks are well mixed.

(c) Add 100 c.c. of distilled water, strain through a sterile cloth.

(c) Pour 10 c.c. each into sterile test tubes, slant, and

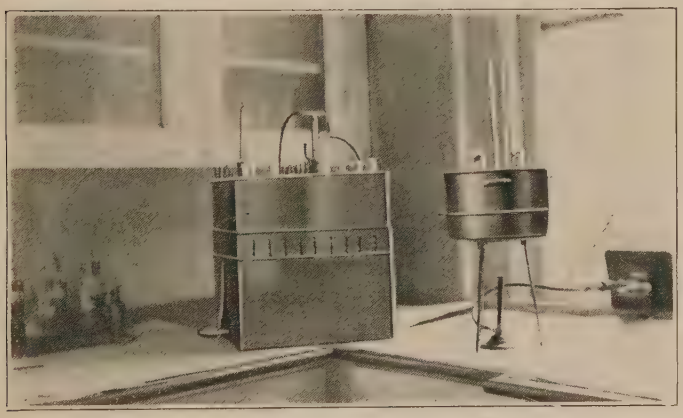


Fig. 20.—Two types of water-baths.

put them into an inspissator, sterilizing at 72° C. for four hours on two days.

(d) On third day raise the temperature to 76° C.

(e) Sterilize in Arnold's sterilizer at 100° C. for fifteen minutes.

Add two or three drops of sterile water to each tube before inoculation.

One of the new media which is excellent is that of Williams and Burdick.*

*Jour. Bacteriol., July, 1916, i.

(a) Dilute the egg whites with 10 parts of distilled water, shake well; pass through a thin layer of cotton, heat to 100° C., filter through cotton.

(b) Yolks are diluted with 10 parts of distilled water, and well stirred. Add 1 c.c. of normal sodium hydroxide solution to 100 c.c. of yolks emulsion. Heat to 100° C., and filter.

(c) 500 grams of finely chopped lean veal are put into 1,000 c.c. of water containing 15 per cent glycerin; put on ice for twenty-four hours, filter, and add 5 grams of sodium chloride, and heat to boiling. Filter and render 1 per cent alkaline (to phenolphthalein).

(d) Put 300 c.c. of 10 per cent egg white solution (obtained in *a*) into a liter flask. Three hundred c. c. of the yolks solution are placed in another liter flask; 400 c. c. of the veal infusion, to which 15 grams of powdered agar are added, are placed in a third liter flask.

(e) Sterilize all three flasks in the autoclave at 15 pounds pressure for fifteen minutes. While hot, pour into the meat infusion flask 1 c.c. of 1 per cent alcoholic solution of gentian violet.

(f) Then pour the contents of this flask into the egg whites flask, and pour into it the contents of the yolks flask. Pour the whole back and forth until thoroughly mixed. Tube and slant for seventy-two hours at the room temperature. Flame the stoppers.

No sterilization is permitted, and for this reason the entire procedure should be carried out under the strictest aseptic precautions.

CHAPTER VI

APPLIED BACTERIOLOGY

Examination of Material from Patients.

1. **Examination of Smears.**—Very frequently it is necessary to examine the discharge from some part of the body; in such case a drop of discharge is placed on a clean sterilized glass slide by means of a sterile swab or platinum loop, and is evenly spread (if the discharge is very "thick" it may be emulsified on the slide with a drop of sterile physiologic 0.8 per cent salt solution). It is then dried in the air, and passed several times through a Bunsen burner flame. Stain by Gram's method.

2. **Making Ordinary Cultures.**—When cultures have to be made from discharge, one must be governed in his choice of media by the organism suspected; if staphylococcus is suspected use meat extract agar, but since streptococcus may also be present and since it does not grow so very well on meat extract media, it is best to use meat infusion agar or broth. If gonococcus is suspected use ascitic fluid salt-free agar, or meat infusion glucose or blood agar, or cornstarch agar.

3. **Anaerobic Cultures.**—At times an anaerobic organism may be suspected, and it is well then to make an anaerobic culture. This consists of making an ordinary culture and placing it in a specially constructed

"anaerobic jar" and incubating it in the usual manner.

If such a jar is not on hand, a 2 per cent glucose meat infusion agar (not slanted) is inoculated by stabbing and about 3 to 5 c.c. of liquid paraffin is poured on the top of the agar.

Another very simple method is as follows: a tube of slanted agar is inoculated in the usual manner, the stop-

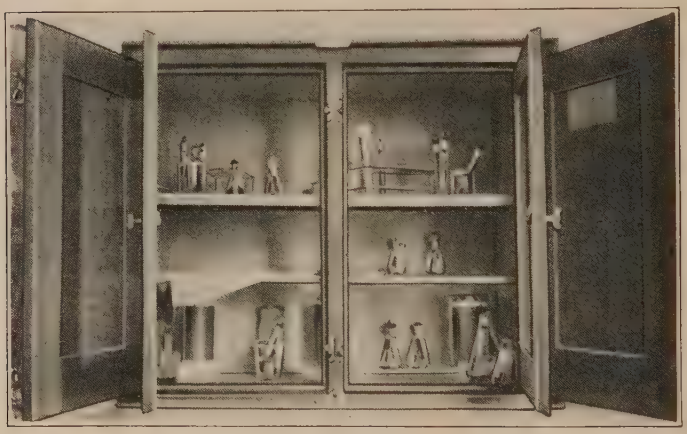


Fig. 21.—The incubator.

per is removed, and the tube is inverted into a beaker containing a gram of dry pyrogallic acid. About 10 c.c. of a 5 per cent solution of sodium hydroxide are then poured into the beaker and this is then covered with a half-inch thick layer of liquid paraffin.

4. **Making Plates.**—If a smear examination or the appearance of the culture suggests that more than one organism is present and it is desirable to isolate them in pure culture, one must "prepare plates": three tubes of agar are melted and cooled to 42° C. A platinum loopful of

pus or other infectious material is transferred to Tube 1 and well mixed by rotating the tube; two loopfuls are transferred from Tube 1 to Tube 2, and five loopfuls from Tube 2 to Tube 3; in this way the bacterial dilution is made progressively higher. The contents of each tube are then poured into each of three Petri dishes, which are numbered to correspond to tubes, and the plates are incubated. Next day the separate colonies are studied in plate No. 3 (containing the least number of colonies), and separate colonies are picked and inoculated on slants, if necessary.

5. **Examination of Peritoneal, Pleural and Pericardial Fluids.**—These should be first centrifugalized and the sediment examined in a smear preparation. Cultures may be made either in a liquid medium by adding a few cubic centimeters of the material to a tube or a flask of the proper medium, or plating on agar.

6. **The examination of cerebrospinal fluid** should be carried out in the same manner as above.

7. **Urine.**—Catheterized specimen of urine should be secured, centrifugalized and smears and cultures made in the usual way.

8. **Feces** is most frequently examined for typhoid bacillus: a small piece is emulsified in a test tube of sterile physiologic salt solution, and dilutions are made into three tubes, as in plating, the contents of each be-being poured in Petri dishes containing Endo's medium; next day a colorless colony is picked and transferred to Russell's double sugar agar tube; after twenty-four hours' incubation if the colony was one of typhoid bacillus, part of the tube will have turned red but no gas bubble will have formed. (See section on Russell's Medium under Culture Media.)

9. **Blood Culture.**—Sterilize the elbow bend with two applications of tincture of iodine, apply a tourniquet half way up the arm, to make the veins stand out prominently, with a sterile all-glass Luer syringe withdraw 5 to 10 c. c. blood.

If typhoid is suspected, pour about 5 c.c. of blood into a flask with bile-peptone-glycerin medium, incubate for 12 hours, and then make transfers to ordinary broth (about 1 to 2 c.c. of bile medium) or make plates on Endo's medium. If septicemia or pneumonia are thought of, pour 1 to 2 c.c. of blood into each of 3 or 4 flasks with glucose meat infusion broth.

10. **Sputum.**—Sputum is usually examined for tubercle bacilli. A thick yellowish piece should be chosen and smeared on a glass slide in a usual way, dried and fixed by heat; it is then stained for tubercle bacillus as described in the section on Staining.

11. **Animal Inoculation.**—Very often when no evidence of tuberculosis can be found on examination of smears or cultures, animal inoculation has to be resorted to.

The fluid to be injected (peritoneal, pericardial, pleural or cerebrospinal fluid) is centrifugalized and the sediment is mixed with a few cubic centimeters of sterile salt solution, and 1 c.c. is injected into a guinea pig intraperitoneally. If sputum is used, a suitable (yellowish) piece is rubbed up with salt solution, instead of centrifugalization. Four weeks later the guinea pig is killed and examined for the evidences of tuberculosis. If the animal dies before the time specified, autopsy is immediately made.

If the guinea pig is x-rayed for thirty seconds before injection ten or fourteen days are a sufficiently long period to wait (as x-raying destroys the lymphoid tis-

sue which protects the animal against tuberculosis, and it is thus rendered more susceptible).

If pneumonia is suspected, one c.c. of blood is injected intraperitoneally into a white mouse, and cultures and smears are made from the peritoneal fluid.

It should be always borne in mind that the final results depend on the care with which all manipulations are made, as the opportunities for contamination are great. Scrupulous care should be exercised in flaming the cotton stoppers before and after opening tubes and flasks with culture media, the platinum needles and loops, etc. Certain procedures can not be explained, but have to be seen in order to be grasped; the pupil can, however, learn at the outset the fact that care in handling the materials is more essential than speed.

SECTION II

SPECIAL BACTERIOLOGY

CHAPTER VII

THE STAPHYLOCOCCUS GROUP

I. Historical

The staphylococcus group were first carefully studied in 1879 by Koch, Ogston, Pasteur and Rosenbach. They are so called because of their appearance in irregular clusters (from Greek, meaning bunch of grapes).

II. Morphology and Staining

It is a coccus, round or spherical, diameter about 0.9 μ. They are usually arranged in clusters, but very young cultures may show diplococci (pairs).

Staphylococci stain with all simple stains, and are Gram positive; they are nonmotile, have no spores, no capsules, no flagella.

III. Cultural Characteristics

Staphylococci grow readily on all plain media; they are aerobes and facultative anaerobes (that is, they may grow without oxygen). They form in 24 hours discrete, golden yellow colonies of various sizes. Gelatin is liquefied. Milk is coagulated and acids (lactic and butyric) are formed.

On sugar media acids are formed but gas is not produced. Yellow pigment is universally formed.

IV. Destruction

Staphylococcus is killed by heating to 58° C. for ten minutes; it is very resistant to low temperatures, and drying is well borne.

Solution of bichloride of mercury 1:1000 destroys

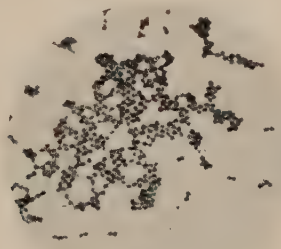


Fig. 22.—Staphylococcus. x 1100 diameters. (Park and Williams—*Pathogenic Bacteria and Protozoa.*)

them in 10 minutes; tincture of iodine is excellent for destroying them.

V. Disease-Producing Properties, Mode of Infection, Disinfection and Prophylaxis.

Different strains vary in virulence. Ordinarily they cause local suppuration—abscesses, boils, carbuncles, etc. They also cause osteomyelitis (inflammation of the bone marrow); if they gain entrance to circulation they may cause septicemia, and abscesses in various organs. Childbirth fever is fairly frequently caused by them. For disinfection see under Destruction. As to prophylaxis, sur-

gical cleanliness is the only efficient means. The nurse should carefully destroy the soiled dressings by burning and disinfect her hands by washing them in 70 per cent alcohol or 1:1000 bichloride of mercury. The room may be fumigated.

VI. Infection and Immunity

Staphylococci produce endotoxins, and in addition to this, hemolysin, which destroys the red blood cells, and

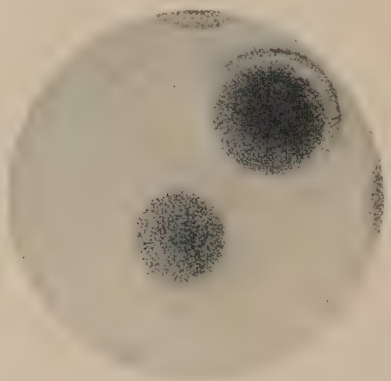


Fig. 23.—Staphylococcus pyogenes aureus. Colony two days' old, seen upon an agar-agar plate. x 40 (Heim). (From McFarland—*Pathogenic Bacteria and Protozoa.*)

leucocidin which destroys the white blood cells (leucocytes).

During the infection agglutinins are produced, bacteriolysins, and opsonins. Phagocytosis is important in staphylococcus infections.

VII. The Varieties of Staphylococci

(a) Staphylococcus pyogenes (pus-producing) aureus (yellow) produces yellow pigment,

(b) Staphylococcus pyogenes albus (white) produces white pigment.

The aureus is the most frequent. The albus causes the stitch abscesses seen after operations.

VIII. Bacteriologic Diagnosis

Bacteriologic diagnosis is easy and rests on the following:

(a) Gram-positive cocci arranged in clusters.

(b) Grow readily on simple (meat extract) media.

(c) Liquefy gelatin, coagulate milk, produce acids, form no gas on sugar media.

IX. Immunity Treatment

None for acute infections. For chronic infections such as continuous abscess formation, persistent pus discharge, etc., a staphylococcus vaccine gives good results. Vaccines are prepared as follows:

A 24-hour-old agar culture of staphylococcus is obtained; about 10 c.c. of sterile salt solution are poured into the culture tube; the growth is gently scraped off with a platinum loop (care being taken not to scrape any particles of agar). The bacterial emulsion is poured into a killing tube (this is an ordinary test tube but with a long drawn out neck) in which 8 to 10 glass beads are placed, the tube is scaled off and shaken for an hour by hand (or for ten minutes in a special "vaccine shaker"); the narrow tip is broken off and a few drops are poured into a sterile watch-glass, the tube is scaled again and placed in a water-bath at 58° C. for one hour, to kill the bacteria; now we proceed to standardize the vaccine; i. e., to determine how many bacteria we have there per cubic

centimeter. With a blood lancet prick a finger and draw blood into a capillary pipette for a distance of one inch (marked with red pencil); a little air is then allowed to enter and then the bacterial emulsion is drawn up to the same mark. We now have equal amounts of blood and bacteria; these are thoroughly mixed by alternate drawing in and out of the contents of the pipette on a watch-glass. Then smears are made of this mixture on a glass slide in the same manner as blood smears; put a large drop of the mixture on a glass slide and with another glass slide resting on the drop make a quick rapid smear. Stain with Wright's stain (see section on Staining); count several fields, counting red blood cells, and bacteria. Let us suppose that we counted altogether 300 red blood cells and 150 bacteria. That means there are twice as many red blood cells as bacteria. Since there are 5,000,000 red blood cells in a cubic millimeter of normal blood, our vaccine (which contains half as many bacteria as there are red blood cells) contains 2,500,000 bacteria per cubic millimeter, or 2,500,000,000 per cubic centimeter. Now if we do not want our vaccine to be so concentrated, we can dilute it with salt solution to the desired concentration.

The vaccine is now taken out of the water-bath, poured into a sterile graduated cylinder, enough salt solution is added to bring it to the desired concentration, and 0.25 per cent of tricresol is added, and the vaccine is allowed to stand at room temperature overnight; next morning aerobic and anaerobic cultures are made, and if, in forty-eight hours, no evidences of contamination are seen, the vaccine is poured into small ampoules and sealed.

X. The Summary of Staphylococcus Group

The staphylococcus is a common organism, found everywhere, especially on human skin, causes localized suppurations, at times septicemia, is Gram-positive, appears in clusters, has no spores, flagella or capsules, is non-motile, grows readily on all ordinary media, is aerobic and facultative anaerobic, produces endotoxin, hemolysin and leucocidin, the infected animal produces bacteriolysins, opsonins, agglutinins; phagocytosis plays an important part in overcoming the infection.

One of the most promising treatments for all infected wounds is that of Carrel-Dakin.

It is somewhat complicated and should be thoroughly explained by one familiar with its use, and, for this reason, is omitted here; briefly stated its essential feature is chloride of lime with sodium carbonate and bicarbonate.

When properly applied by Carrel-Dakin's method, it is the most efficient treatment of infected wounds yet devised.

CHAPTER VIII

THE STREPTOCOCCUS GROUP

I. Historical

The first studies on streptococci were made by Klebs, Koch, Pasteur and Ogsten.

II. Morphology

Streptococci are small spherical organisms, about 0.7 μ in diameter. They usually are arranged in chains, whence their name (from Greek *streptos*, meaning twisted). The chains are usually longer in the strains just isolated from animal tissues than in those grown on artificial culture media. They also appear to grow in longer chains in liquid than in solid media.

The streptococci are nonmotile, have no flagella, no spores, no capsules, although there is a strain of streptococcus which regularly possesses capsules, the so-called streptococcus pyogenes (pus-producing) capsulatus, but today this organism is regarded as being one of the strains of pneumococcus and is called pneumococcus capsulatus (see section on Pneumococcus).

Streptococcus is Gram-positive.

III. Cultural Characteristics

Streptococcus grows slowly on ordinary meat extract media, but luxuriantly on the "enriched" media, such as meat infusion media, especially when glucose or as-

citic fluid has been added. On blood agar plates streptococci colonies form a wide zone of hemolysis. Gelatin is not liquefied. Sugars are fermented and acid is produced. Milk is coagulated in three to four days.

It is aerobic and facultatively anaerobic. It does not grow below 15° C. or above 42° C.

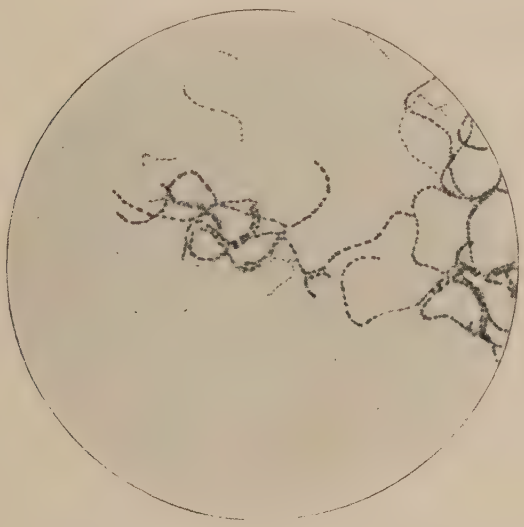


Fig. 24.—Streptococcus pyogenes. Film preparation of a broth culture. x 1500. (Hewlett—*Manual of Bacteriology.*)

IV. Destruction

Heating at 60° C. for one hour will kill streptococci, 5 per cent carbolic acid solution and 1:1000 bichloride of mercury kills them in a few minutes. They keep well on defibrinated blood in ice box. Drying destroys them in ten hours.

V. Disease Production, Mode of Infection, Disinfection and Prophylaxis

Streptococci are widely distributed in nature, but usually in association with man, they are found in soil, water and milk. Some very severe epidemics of "streptococcus sore throat" have been caused by the use of infected milk.

In human beings they cause both local suppurations like staphylococci and also septicemia ("blood poisoning "of the laity); infections develop very rapidly from twelve hours on, and if it becomes general, produces endocarditis (inflammation of the lining of the heart), meningitis (inflammation of the lining of the skull), childbirth fever, acute rheumatism, sore throat (tonsillitis), osteomyelitis (inflammation of the bone-marrow), pneumonia ("septic" pneumonia); they often accompany other infections, e. g., diphtheria. They invariably enter through the skin; the prevention is surgical cleanliness, disinfection, and fumigation.

VI. Mechanism of Infection and Immunity

Streptococci produce endotoxins, also hemolysin (which destroy red blood cells).

Immune bodies produced in infected animals are agglutinins, bacteriolysins and opsonins; phagocytosis is important in combating the infection.

VII. Bacteriologic Diagnosis

Gram-positive cocci, usually arranged in chains, grow slowly or not at all on meat extract media, but very well on "rich" media, cause hemolysis on blood agar plates. If confused with pneumococci, differentiation may be difficult; see section on Pneumococcus.

VIII. Immune Treatment

In acute infections serums from animals immunized with streptococcus vaccine has been, at times, very helpful. The vaccine is of not much benefit. In chronic infections the vaccine is, at times, very useful. The vaccine is prepared as the staphylococcus vaccine is.

IX. Summary of Important Characteristics

Gram-positive cocci, arranged in chains, nonmotile, no flagella, no spores, no capsules, grow poorly on ordinary media, well on "rich" media, cause hemolysis on blood agar plates; cause either local suppuration or systemic infection; very dangerous organism, enters through the skin, produces an endotoxin and a hemolysin.

CHAPTER IX

THE PNEUMOCOCCUS GROUP (DIPLOCOCCUS PNEUMONIÆ, DIPLOCOCCUS, LANCEOLATUS—"LANCE-SHAPED")

I. Historical

Pasteur isolated the organism from the saliva in 1881; at the same time, quite independently, it was discovered by the American bacteriologist, Sternberg (late Surgeon-General, United States Army). Fraenkel and Weichselbaum in 1886 definitely described it as the cause of pneumonia, in 90 per cent of cases.

II. Distribution

It is widely distributed, in close association with man.

III. Morphology

Through the brilliant work of Cole, Dochez, Avery and other workers at the Rockefeller Institute for Medical Research in New York, we now know that there are at least four different strains of pneumococci, which may be recognized by serologic methods (agglutination and precipitin reaction), but morphologically can not.

Pneumococcus is a lance-shaped, oval organism generally occurring in pairs (in very young cultures not uncommonly it is seen in chains) of varying sizes; it is invariably encapsulated when freshly isolated from animal tissues, but readily loses the capsule on pro-

longed cultivation, which suggests that the latter protects the organism; it is nonmotile, has no flagella, no spores; is Gram-positive, (the Type III pneumococcus capsule is very large and may be seen without using the special capsule stain) the capsulated strain is always more virulent. (See Fig. 2.)

IV. Cultural Characteristics

Pneumococci do not grow on meat extract media; the best media are those which contain meat infusion and rabbit's defibrinated blood; on such media colonies are small, discrete and greenish.

Gelatin is not liquefied, milk is coagulated and acid is produced. Among sugars fermented is inulin—this is very important in differentiating the pneumococcus from streptococcus because the latter does not ferment inulin; bile dissolves pneumococci, but does not dissolve streptococci—another important differentiating point, on blood plates it does not cause hemolysis.

Pneumococcus grows equally well with or without oxygen.

V. Destruction

Pneumococcus does not grow below 25° C. or above 41° C. Heating to 52° C. for ten minutes kills them, as do most of the ordinary chemical disinfectants. Even on the most favorable media it dies within a few days.

The best way to preserve pneumococci is to grow them on defibrinated rabbit's blood agar and keep the cultures in the ice box, or keep them in dried spleens of infected white mice, which should be kept in sealed tubes on ice; when culture is needed, spleen is rubbed with a little broth, the emulsion is injected into a white mouse,

and pneumococci are recovered from the fluid in the peritoneal cavity.

VI. Disease Production, Mode of Infection, Disinfection and Prophylaxis

In addition to causing about 90 per cent of all cases of pneumonia, pneumococci may cause inflammation of bronchi, nose, throat, ear, meningitis, peritonitis (in young children I have seen two such cases), peri- and endo-carditis (inflammation of the sac and the lining of the heart), etc. Many normal persons harbor pneumococci in their mouth and throat. The pneumococci almost invariably enter the body through the respiratory tract.

Disinfection and prophylaxis resolves itself in disinfecting the mouth and throat, isolation of the patient, avoiding to have patient cough in one's face, etc.; fumigation of room should follow the removal of the patient.

VII. Mechanism of Infection and Immunity

Pneumococcus produces an endotoxin; the antibodies formed by the infected animal are bacteriolysins, agglutinins, precipitin, and opsonins, phagocytosis always plays a part in recovery, and marked leucocytosis (increase of white blood cells up to 40,000 per cubic millimeter) is very frequently seen.

VIII. Bacteriologic Diagnosis

The bacteriologic diagnosis is made on the Gram-positive, capsulated diplococci (pairs), growing only on "rich media." If necessary white mice should be injected and autopsy will reveal pneumonia. The organ-

ism which is most likely to be confused with pneumococcus is streptococcus, the following points should be borne in mind.

Pneumococcus	Streptococcus
Almost always capsulated.	Almost never capsulated.
Soluble in bile.	Insoluble in bile.
Ferments inulin.	Does not ferment inulin.
No hemolysis on blood plates.	Hemolysis present.

Finally, agglutination test will not only enable one to make a definite diagnosis, but, in cases of pneumococcus, will identify the type of pneumococcus.

IX. The Different Types or Strains of Pneumococci

As has been mentioned above there are at least four types of pneumococcus:

Type I causes pneumonia in 33 per cent of cases.
Type II causes pneumonia in 31 per cent of cases.
Type III causes pneumonia in 12 per cent of cases.
Type IV causes pneumonia in 12 per cent of cases.

Mortality caused by the different types also differs:

Type I is fatal in 25 per cent of cases.
Type II is fatal in 32 per cent of cases.
Type III is fatal in 45 to 50 per cent of cases.
Type IV is fatal in 16 per cent of cases.

The agglutination test consists in mixing the isolated organisms with the serum of an animal injected with the different types, and observing where agglutination of bacteria takes place. For complete technic the reader should consult the monograph on *Acute Lobar Pneumonia* by Cole and his coworkers (The Monograph No. 7 of the Rockefeller Institute for Medical Research).

Recently Krumwiede* of New York has described a much simpler precipitin test.

X. Immune Treatment

The various vaccines and immune serums up to date are not of much value, but the serum prepared recently by Cole for Type I pneumococcus infection is very efficient and should be tried in all cases where the Type I has been identified.

XI. Summary of Important Characteristics

A Gram-positive, capsulated organism, occurs in pairs, grows only on "rich" media, causes no hemolysis on blood plates, but forms greenish colonies, ferments inulin, is bile soluble; there are four types of strains, the Type III being the most dangerous but occurring in the smallest number of cases; serum treatment is efficient for Type I; produces endotoxin.

*Jour. Am. Med. Assn., 1918.

CHAPTER X

THE MENINGOCOCCUS AND PARAMENINGOCOCCUS GROUP (MENINGOCOCCUS INTRACELLULARIS MENINGITIDIS)

Meningitis (the inflammation of the brain coverings) is caused in 70 per cent by the meningococcus, in 20 per cent by pneumococcus, and in 10 per cent by staphylococci, streptococci, Bacillus tuberculosis, Bacillus influenzæ (grippe), etc.

I. Historical

Meningococcus was first observed in 1884 by Marchiafava and Celli, and thoroughly studied in 1887 by Weichselbaum.

II. Morphology

Meningococci occurs within or without the pus cells when isolated from the cerebrospinal fluid; they usually occur in pairs, like pneumococci do; they vary in size; they are not motile, have no flagella, no spores, no capsules; they stain readily with or without ordinary stains and are Gram-negative.

III. Cultural Characteristics

Meningococci do not grow well on ordinary meat extract media, but grow easily on meat infusion media; milk is not coagulated, gelatin is not liquefied. Meningococcus is an aerobe, the growth not taking place below 25° C., or above 42° C.

IV. Destruction

Sunlight and drying kills the meningococci within twenty-four hours; they are extremely sensitive to heat and cold and are most readily destroyed by highly diluted solutions of the common disinfectants.

V. Disease Production, Mode of Infection, Disinfection and Prophylaxis

Meningococcus causes meningitis; it is usually ac-

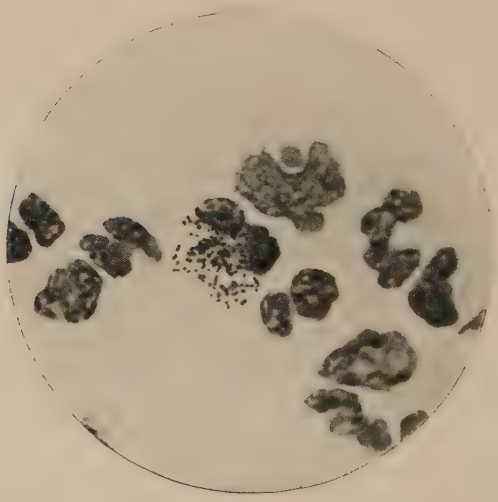


Fig. 25.—The meningococcus. Smear of cerebrospinal fluid. x 1000. (Hewlett—*Manual of Bacteriology.*)

cepted that meningococci first lodge in the nose, whence they enter the skull either directly (through the so-called cribriform plate of the ethmoid bone) or indirectly through the lymph vessels.

The disease is transmitted directly from one human being to the other, as the low resistance of the meningo-

coccus and its susceptibility to drying precludes its being found at large in nature. Coughing, sneezing, the use of infected handkerchief, etc., is sufficient to infect.

Patients should be isolated and quarantined, the nurse should change her outer clothing before mingling with other people, she should carefully disinfect her hands and hair and spray her nose with some silver preparation (argyrol, 10 per cent); the same thing applies to the patient before he is discharged.

VI. Mechanism of Infection and Immunity

Bacteriolysin and agglutinin are produced; meningococcus produces endotoxin.

VII. Bacteriologic Diagnosis

The bacteriologic diagnosis is made on finding Gram-negative cocci, arranged in pairs, both within and without the pus cells, on the fact that the organisms grow only on meat infusion media, on the source of the organism (the cerebrospinal fluid), and if necessary, on agglutination.

VIII. Immune Treatment

To Flexner, of the Rockefeller Institute for Medical Research of New York, belongs the credit of preparing the first successful antimeningitic serum (from the horse), which has reduced the mortality of this dreadful disease from 70 to 80 per cent to 15 to 20 per cent.

IX. Summary of Important Characteristics

A Gram-negative organism, occurring in pairs, growing only on meat infusion media, no spores, no flagella, no

capsule; causes meningitis, produces endotoxin; patients should be isolated and strictest attention should be given to disinfection; serum treatment is the only efficient treatment.

The parameningococci or pseudo- (false) meningococci resemble the meningococci in everything except in not agglutinating in the same way (that is, they agglutinate in much lower dilutions).

CHAPTER XI

GONOCOCCUS

The gonococcus, also called diplococcus gonorrheæ, causes gonorrhea, a highly contagious disease.

I. Historical

It was discovered by Neisser in 1879.

II. Morphology

It occurs in pairs, is Gram-negative, the pairs are characteristically flattened along the facing surfaces—"biscuit" shaped; the gonococci are always found within the pus cells (leucocytes). They are nonmotile, have no flagella, no spores, no capsules.

III. Cultural Characteristics

Gonococci grow only on ascitic fluid agar, cornstarch agar, and also glycerin—or sugar ascitic fluid agar. It is an aerobic organism, and does not grow below 30.5° C. The colonies are extremely delicate, grayish little spots.

IV. Destruction

Gonococci possess very slight resistance to heat and light, exposure to 42° C. for ten minutes kills them; they are, however, very resistant to drying.

Most of the common disinfectants, even in high dilutions, kill the gonococci readily, especially the silver

salts, which fact explains the universal use of the various silver preparations in the treatment of this disease.

V. Disease Production, Mode of Infection, Disinfection and Prophylaxis

At first the inflammation caused by gonococcus is strictly local (urethritis, inflammation of urethra), sometimes, if the discharge is carried to the nose or eye, the

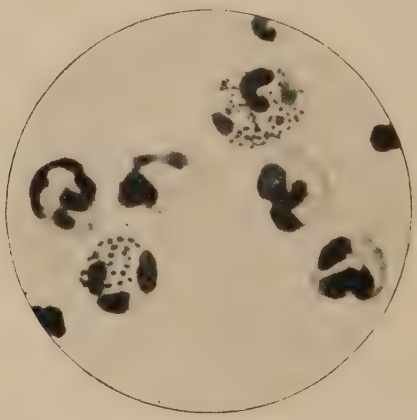


Fig. 26.—The gonococcus. Smear of gonorrhea pus. x 1500. (Hewlett—*Manual of Bacteriology.*)

inflammation is produced in these parts. Gonorrhea is always produced by contact.

After the acute stage, gonococci frequently cause rheumatism, endocarditis (inflammation of the lining of the heart), and valvular heart disease. Babies born of mothers affected by gonorrhea, if not properly taken care of, develop blindness (gonorrheal ophthalmia of the newborn); it is, therefore, almost compulsory now to put one or two drops of a 2 per cent solution of silver nitrate into baby's eyes at birth, and this simple prevention

known as Credé's treatment has practically eliminated this babies' scourge.

The patients should be isolated, and everything touched by them should be most scrupulously disinfected; they should be warned of the danger of infecting their eyes, and the nurse should be extremely careful in disinfecting her hands and had best put a drop of a 10 per cent solution of agyrol in her eyes once a day.

VI. Mechanism of Infection and Immunity

Gonococci produces endotoxins; the immune bodies concerned in recovery are bacteriolysins, opsonins (phagocytosis is very important).

VII. Bacteriologic Diagnosis

Bacteriologic diagnosis is made on finding a Gram-negative diplococcus, biscuit shaped, and within the pus cells.

VIII. Immune Treatment

The gonococcus vaccine is used only in chronic cases, and in these gives very good results.

IX. Summary of Important Characteristics

Gram-negative, biscuit shaped, diplococci, found within pus cells, no spores, no flagella, no capsules, grow only on ascitic fluid agar, cornstarch agar, or other "rich" media, cause gonorrhea, sometimes rheumatism and heart disease, produce endotoxin; the vaccine treatment well worth trying in chronic cases.

CHAPTER XII

MICROCOCCUS CATARRHALIS AND GRAM-NEGATIVE COCCI

I. Micrococcus Catarrhalis

This is an organism found in inflammation of upper respiratory tract. Its significance is unimportant except that being a Gram-negative diplococcus, it can not be differentiated in its appearance from gonococci and meningococci; from gonococcus it differs by growing on simple meat extract media. It is much more difficult to differentiate it from meningococcus, and yet it is especially important as both are usually found in the nose. It grows much better on simple media than the meningococcus, its colonies are coarse while those of meningococcus are very fine, and micrococcus will grow below 25° C., while the meningococcus will not.

II. Gram-negative Cocci

Gram-negative cocci producing green pigment can be separated from the gonococcus and meningococci by sugar fermentation and agglutination.

CHAPTER XIII

THE COLON-TYPHOID-DYSENTERY GROUP
B. COLI COMMUNIS

The reason all these bacteria are usually grouped together although they produce quite different diseases, is that, morphologically (that is, by their appearance in stained preparations), they are almost indistinguishable, and in diagnosis our reliance is placed on cultural characteristics and agglutination.

I. Historical

The colon bacillus or, to give it its full name, bacillus coli communis (which means the common bacillus of colon —the large intestine), was first described by Buchner in 1885, and thoroughly studied by Escherich in 1887.

II. Morphology

Colon bacillus is a short, plump, rod-like organism, $\frac{1}{3}$ μ long, usually occurring singly, is Gram-negative, has no spores, no capsules, is motile, and has flagella. (See Fig. 3.)

III. Cultural Characteristics

It is aerobic organism, grows well on simplest media, i. e., meat extract agar and broth; it grows at any temperature between 20° C. and 40°C. It does not liquefy gelatin, forms indol on peptone media, coagulates milk and forms acid in it; as to sugar fermentation, it both

ferments and produces gas on dextrose (glucose, or grape sugar), lactose (milk sugar), maltose (malt sugar), levulose (fruit sugar), galactose and mannite, but neither ferments nor produces gas on saccharose (cane sugar).

IV. Destruction

Colon bacillus is readily destroyed by heating to 58° C. for ten minutes, cold affects it less, and ordinary disinfectants readily kill it. Excessive alkaline reaction inhibits its growth.

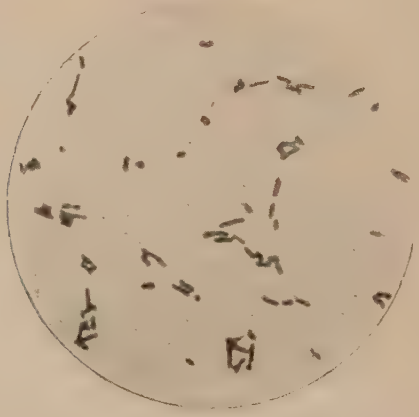


Fig. 27.—Bacillus coli. Film preparation from a pure culture. x 1000. (Hewlett—*Manual of Bacteriology.*)

V. Disease Production, Mode of Infection, Disinfection and Prophylaxis

The colon bacillus is normally found in the intestine of both man and animal, also occasionally found in soil, water and milk. Its presence in nature usually means contamination from animal or human sources. In the human intestine it is found in greatest numbers near the junction of the small and large intestine.

Although the colon bacillus is a normal inhabitant of the human intestine and a useful one, too, since its presence seems to inhibit the growth of many harmful putrefactive bacteria, yet at times it gives rise to various diseases; as a rule any enfeebled condition seems to make it easier for the colon bacillus to become pathogenic; among the various diseases caused by this organism are the various diarrheas—cholera infantum and cholera nostras, occasionally peritonitis (but usually accompanied by other organisms); very frequently it is found in inflammatory conditions of the liver, gall bladder, and appendix; one of the most frequent conditions caused by the colon bacillus is the inflammation of the urinary bladder (cystitis) and pyelitis (inflammation of the basin of the kidney).

It is often found in the abscesses within the abdominal and pelvic cavities.

No special precautions are called for in handling the colon infections, beyond following the usual hygienic and sanitary rules.

Bacillus coli communior differs from the colon bacillus in fermenting and producing gas on saccharose in addition to all other sugars.

VI. Mechanism of Infection of Immunity

The colon bacillus produces an endotoxin, and the antibodies produced by the animal during the colon infections are the bacteriolysins, precipitins and agglutinins.

VII. Bacteriologic Diagnosis

The differentiation of the colon, typhoid, paratyphoid and dysentery bacilli will be discussed fully in the chapter on Typhoid Bacillus.

VIII. Immune Treatment

Vaccine treatment of colon infection of the kidney and urinary bladder is very successful.

IX. Summary of Important Characteristics

A Gram-negative bacillus, no spores, no capsule, motile, has flagella, grows readily on common media, both ferments and produces gas on all common sugars except saccharose; is found normally in human and animal intestine, causes infections of the gall bladder, urinary bladder and kidney, also found in several infections of the pelvis and abdominal cavity.

CHAPTER XIV

BACILLUS TYPHOSUS

I. Historical

Bacillus typhosus was first thoroughly studied by Eberth in 1880. Gaffky first isolated them in pure culture in 1884.

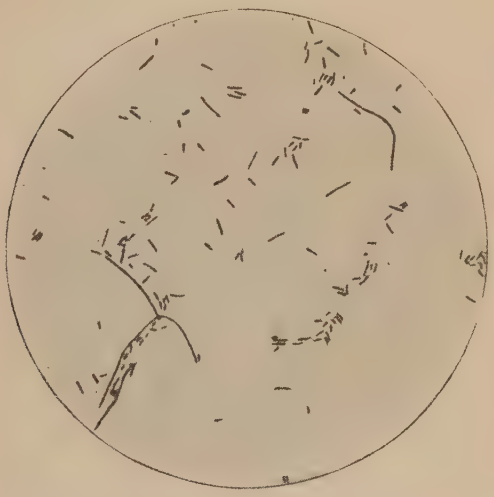


Fig. 28.—Bacillus typhosus. Film preparation of a pure culture. x 1500. (Hewlett—*Manual of Bacteriology.*)

II. Morphology

The morphology of bacillus typhosus resembles that of the colon bacillus and other members of the group very closely, with the exception that it is more slender. Is

Gram-negative, is motile, has flagella, has no capsules or spores.

III. Cultural Characteristics

The cultural characteristics are the same as those of the colon bacillus except as regards some sugars and milk, as shown in the following table:

TABLE OF REACTIONS ON SUGAR MEDIA AND MILK BY THE VARIOUS MEMBERS OF THE TYPHOID-COLON-DYSENTERY GROUP

NAME OF BACTERIUM	DEXTROSE (GRAPE SUGAR)	LEVULOSE (FRUIT SUGAR)	GALACTOSE	MANNITE	MALTOSE (MALT SUGAR)	LACTOSE (MILK SUGAR)	SACCHAROSE (CANE SUGAR)	MILK
B. Fecalis Alcaligenes	——	——	——	——	——	——	——	No coagulation
B. Dysenteriæ (Shiga type)	A	A	A	——	——	——	——	No coagulation
B. Dysenteriæ ("Y" type)	A	A	A	A	——	——	——	No coagulation
B. Dysenteriæ (Flexner type)	A	A	A	A	A	——	A	No coagulation
B. Typhosus	A	A	A	A	A	——	——	No coagulation
B. Paratyphosus	AG	AG	AG	AG	AG	——	——	No coagulation
B. Coli Communis	AG	AG	AG	AG	AG	AG	——	Coagulation takes place
B. Coli Communior	AG	AG	AG	AG	AG	AG	AG	Coagulation takes place

(After Hiss and Zinsser.)

A=Fermentation with acid production, A G=Fermentation with both acid and gas production, —— means no fermentation.

IV. Destruction

Bacillus typhosus is an aerobe and a facultative anaerobe; grows between 15° C. and 42° C., but, like the rest of bacteria, grows best at 37° C. Heating for ten min-

utes at 56° C. destroys it. On artificial culture it will remain for many months, if sufficient moisture is supplied. It will keep alive for many months on ice and in water. It is killed somewhat less readily by ordinary disinfectants than most of the bacteria, but 1:500 solution of bichloride of mercury will kill it in a few minutes.

V. Disease Production, Mode of Infection, Disinfection and Prophylaxis

Bacillus typhosus causes typhoid fever. During the first week or ten days the bacilli are found in the blood and "rose spots" (the rash of typhoid fever). Quite often the bacilli, after the patient recovers lodge in the gall bladder whence they are continually discharged into the intestine and thus appear in the feces, in this way while the patient is well and immune, he may become the so-called "bacillus carrier" and be a temporary or permanent source of danger to the community. For this reason all patients who have recovered from typhoid fever, should have their stools examined, and, if typhoid bacilli be found, should be warned to take special precautions about disinfecting the stools, etc.

In about 25 per cent of cases the typhoid bacilli may be cultured from the urine.

Very frequently the presence of this bacteria in the gall bladder causes its inflammation (cholecystitis) and the gallstones.

Suppurations may be caused by the typhoid bacillus especially in the long bones and in the ribs or spine and, not infrequently, meningitis.

Typhoid fever is always with us, but from time to time severe epidemics take place.

Practically all cases of typhoid fever are contracted

through swallowing the bacilli either directly in food, water, ice, milk, vegetables, etc., or indirectly by soiling fingers with patient's excreta, or using the same utensils, glassware, etc.

The prophylactic measures, therefore, consist of thorough disinfection of stools, urine, bed linen, etc., as described in detail in the chapter on Practical Disinfection; since typhoid fever is essentially a "water borne" disease, communities should guard the purity of their water supply, most effectively by having a modern filtration plant, and removing the source of contamination and sewage pollution; milk supply should be closely inspected and flies should be destroyed as they very often contaminate our food—screening is necessary.

During a typhoid epidemic drinking only boiled water is a wise precaution. The nurse attending a typhoid patient should receive prophylactic typhoid vaccination, which is the best prevention.

VI. Mechanism of Infection and Immunity

Typhoid bacillus produces an endotoxin; the antibodies produced are bacteriolysins, precipitins and agglutinins, one attack usually renders a person immune to subsequent attacks.

As regards the agglutinins, a few words must be said here about this:

The blood of most of normal individuals has slight agglutinating property, but this is enormously increased during and following the diseases or by artificial immunization (vaccine). From the fact that the typhoid, paratyphoid and the colon bacilli are all closely related to each other both morphologically (all being Gram-negative, motile, flagella-possessing bacilli) and in their reac-

tions on some sugars, it is not surprising to learn that the agglutinins produced by the blood in response to these bacteria are also evidently related to each other; that is to say, agglutinins caused by injection of typhoid bacilli will agglutinate, not only typhoid bacilli, but also those of the paratyphoid and colon bacilli although to a much lesser extent; those produced by the injection of the colon bacillus will cause agglutination—to a much lesser degree—of the typhoid and paratyphoid bacilli, etc.

The expression "to a much lesser degree" means in a higher concentration; i. e., in lower dilution; for example, the agglutinins caused by injection of the typhoid bacilli will agglutinate the typhoid bacilli in the dilution of the blood serum in salt solution of 1:10,000, while they will only agglutinate the colon and the paratyphoid bacilli in dilution of 1:200, etc.

When the injection of an animal with one bacterium results in the production of agglutinins not only for that organism but also to a lesser extent for other bacteria, closely related to it (as when the blood serum of a rabbit injected with the typhoid will agglutinate not only the typhoid bacilli but also, to a lesser degree, i. e., in lower dilutions, the paratyphoid and colon bacilli)—such agglutinins clump or agglutinate the entire group, although to a varying extent. The specific agglutinins (that is, those against the bacteria injected) are called "major" agglutinins and those against other members of the groups are called "minor" agglutinins; thus, if a rabbit is injected with typhoid bacilli, he will produce the major or typhoid agglutinins, and minor or paratyphoid and colon agglutinins.

This is shown in the accompanying table, which is copied from the actual work done in our laboratories.

(The rabbit was injected with typhoid bacilli, and his blood serum showed the following agglutination, in various dilution of blood serum with salt solution.)

Dilution	1:100	1:200	1:500	1:1000	1:3000
Typhoid Bacillus	+	+	+	+	+
Paratyphoid Bacillus	+	+	+	—	—
Colon Bacillus	+	—	—	—	—

+=Agglutination takes place.
—=Agglutination does not take place.

This table shows that the blood serum of a rabbit injected with the typhoid bacilli had developed (1) the major or typhoid agglutinins which agglutinated the typhoid bacilli in a dilution as high as 1:3000, and (2) minor paratyphoid agglutinin which agglutinated the paratyphoid bacilli in dilution up to 1:500, and (3) the minor colon agglutinin which agglutinated the colon bacilli in dilutions up to 1:100; this also shows that the paratyphoid bacillus are more closely related to the typhoid bacillus than the colon bacillus is. The technic of agglutinations is given in the next paragraph.

VII. Bacteriologic Diagnosis

Whether the culture is obtained from the blood or feces, plates must be made, as explained in the section on General Bacteriology. My method is to make the plates on Endo's medium (see section on Culture Media) where the colon bacillus colonies are red and the typhoid colonies are colorless; from such a plate a colorless colony is picked and transferred to a Russell's double sugar medium (see the section on Culture Media), inoculating both the surface and by stabbing; by referring to the table of sugar fermentations, it will be understood that if the culture is typhoid, only a part of the tube will turn from blue to red (because the typhoid bacilli act only on glucose, producing acid gas only), and no

gas bubbles will be seen (because the typhoid does not form gas on glucose); if the culture is paratyphoid, again only part of a tube will turn to red but gas bubbles will also be seen in the red part only (because the paratyphoid bacilli form both acid and gas on glucose and do not ferment lactose); finally if the culture should be contaminated with colon bacillus, the culture tube will be red and gas bubbles will be seen everywhere (because the colon bacillus produces both acid and gas on both the glucose and the lactose). If this is insufficient agglutination should be done as follows:

Four rows of Wassermann test tubes (4 x ½ inch) should be arranged, each row containing 10 test tubes; the first row is labeled "typhoid," the second "paratyphoid A," the third "paratyphoid B," and the fourth "colon." In the first tube of each row put 1.8 c.c. of sterile physiologic (0.8 per cent) salt solution, in all other tubes put 1 c.c. of salt solution; then into the first tube put 0.2 c.c. of the typhoid immune serum of known agglutinating properties (prepared by injecting the rabbit three or four times with typhoid vaccine); now we have in this tube a 1:10 dilution of typhoid serum (1.8 c.c. salt solution and 0.2 c.c. of serum); take one c.c. out of this tube and put it into the next tube of the "typhoid" row; we had before in that second tube 1 c.c. of salt solution, now we have 2 c.c. of fluid, one c.c. being salt solution, the other c.c. being 1:10 dilution of the immune typhoid serum from the first tube, so that the second tube contains the serum in dilution 1:20; repeat this process until the last tube is reached, and throw away the last c.c.; all tubes now contain 1 c.c. of fluid, but the dilutions are all doubled, the amount of serum being halved as we go from one tube to another: 1st tube, 1:10; 2nd,

1:20; 3rd, 1:40; 4th, 1:80; 5th, 1:160; 6th, 1:320; 7th, 1:640; 8th, 1:1280; 9th, 1:2560; 10th, 1:5120. Make similar dilutions in the other three rows, putting 0.2 c.c. of immune paratyphoid A serum in the paratyphoid A row, 0.2 c.c. of immune paratyphoid B serum in the paratyphoid B row, etc. Then take a platinum loop, flame it, and having added about 5 c.c. of salt solution to the culture tube containing the bacteria you want to identify, scrape off the growth, shake well by rotating the tube in your hands, and add to every tube in each row 5 drops of the bacterial emulsion (to make the entire procedure safe, heat the emulsion for 1 hour at 58° C.; this will kill the bacteria but will not interfere with the agglutination); incubate at 37.5° C. for one hour and then examine the tubes; the row which contains tubes in highest dilution corresponds to the infection of the patient, that is, if the typhoid row (which contains tubes with the typhoid immune serum) shows agglutination in a tube with higher dilution than in any other row—your culture is typhoid.

Since, however, typhoid bacilli may be recovered from patient's blood only during the first ten days of the disease, and the patient may be brought to the hospital later, the blood cultures may prove to be "negative," that is the bacilli will not be found. In such cases, patient's blood is removed in a test tube (about 2 to 3 c.c.), and Widal test is made. This test is agglutination, but just the opposite to that made on the culture: there we worked on an unknown culture with known immune serums, while now we will work on an unknown serum with known cultures: four rows of 10 tubes in each prepared as before, the first tube in each row containing 1.8 c.c. of salt solution, the others containing 1 c.c. of salt solution; the rows again are labeled as before "typhoid," "paratyphoid A," "paratyphoid B," and "colon." Now

in *each* first tube put 0.2 c.c. of the patient's serum, and continue the dilution along as described above, i. e., take 1 c.c. from first tube to the second, from the second to the third, and so on, *in each row;* throw away the last c.c. (that is, from the 10th tube). Now to each tube in the "typhoid" row add 0.5 c.c. of typhoid culture with salt solution and killing it with 2 per cent of formalin, to each tube of the paratyphoid A row add 0.5 c.c. of the paratyphoid A culture, and so on. Incubate for one hour at 37.5° C. and examine. The row containing the tube with the highest dilution gives you the nature of the patient's disease.

Sometimes the so-called "microscopic Widal test" is made; this consists of making two or three dilutions (usually 1:20, 1:40, and 1:80) with patient's serum, and a drop of each is mixed with a drop of typhoid emulsion, and placed on a cover-glass which is then placed over a "hanging drop" slide, as described elsewhere, after one hour the slide is examined to see if motility has ceased and clumping has taken place. This is a convenient quick test, but not nearly so comprehensive as the preceding method.

VIII. Immune Treatment

Whether the use of the typhoid vaccine is to be adopted for the treatment of typhoid fever is rather doubtful; but the use of such vaccine for the prevention of typhoid fever is not only an established fact but constitutes one of the most glorious achievements in bacteriology and prevention of infectious diseases, and is one of the greatest medical services rendered the world by the English and American scientists.

In England, the preventive use of vaccine was brought to perfection by Sir Wright, and in 1907-8, Major (now Colonel) Russell of the United States Army Medical

Corps, was sent to England to study the question. Upon his return the typhoid vaccine was adopted for the United States Army and Navy, and, subsequently, throughout the entire civilized world.

All persons should be vaccinated with typhoid vaccine as it confers almost absolute immunity for at least three years.

As it is now put up at the United States Army Medical School, it is a "triple" vaccine, containing the typhoid, the paratyphoid A and paratyphoid B bacilli, 1,000 million of each to each c.c.

The preparation of the vaccine is practically the same as that described for staphylococcus vaccine.

IX. Summary of Important Characteristics

The important characteristics are the same as those of colon, except that it produces acid only (as gas) on all common sugars except lactose and saccharose. Causes typhoid fever, is essentially a "water-borne" disease; the preventive vaccination is of tremendous importance.

The disease is of such practical importance to the nurse that no attempt is made to summarize this chapter, and the reader is advised to read carefully the entire chapter.

CHAPTER XV

THE B. PARATYPHOSUS A AND B. THE B. FECALIS ALCALIGENES. THE B. PROTEUS. DYSENTERY BACILLI

B. Paratyphosus A and B

The paratyphoid bacilli cause a disease which simulates typhoid fever so closely that no differentiation is possible from the symptoms (unless it be the milder form of the disease when caused by the paratyphoid bacilli), and the diagnosis is usually made from the cultural study and agglutination (see the table of Sugar Fermentations and the Bacteriologic Diagnosis of Typhoid Fever). Of the two types of the paratyphoid bacilli, B is of much more importance than A.

B. Fecalis Alcaligenes

This organism is of little pathogenic importance and is only interesting because of ease with which it may be confused with bacillus typhosus.

It is frequently found in normal intestine and feces. It is differentiated from typhoid, colon, and paratyphoid bacilli by the fact that it does not ferment any of the common sugars.

Bacillus Proteus

Bacillus proteus possesses slight pathogenic properties, but occasionally it may cause infections of the bladder and abscesses.

It is very frequently encountered in the laboratory work and may be confused with typhoid bacillus, but cultural studies and agglutination make its differentiation easy.

The Dysentery Bacilli

Dysentery is an infectious disease characterized by severe diarrhea.

Fig. 29.—Dysentery bacilli. x 1000. (Park and Williams—*Pathogenic Bacteria and Protozoa.*)

The first to discover the organism was a Japanese bacteriologist, Shiga (1898).

There are three organisms—the Shiga type, the "Y" (Hiss-Russell) type, and the Flexner type. All three types morphologically resemble each other and the rest of the typhoid-colon-dysentery group.

The differentiation and the diagnosis is made on cultural (sugar fermentation) characteristics and agglutination, as described in Chapters XIII, XIV and XV.

CHAPTER XVI

BACILLUS MUCOSUS CAPSULATUS (FRIEDLÄNDER'S OR PNEUMO-BACILLUS)

This organism first described by Friedländer in 1882 causes about 5 per cent of all cases of pneumonia; such pneumonia is extremely dangerous.

It is occasionally found in ulceration of the mouth, nasal catarrh, ozena (fetid nasal catarrh), empyema (pus in the pleural cavity), and spinal fluid. The author once recovered it from a gall bladder infection and from blood in subsequent septicemia.

Friedländer's bacillus is short and plump, has no spores, no flegalla, is nonmotile, has very large capsule; it is Gram-negative, and grows well on all ordinary meat extract media, producing peculiar, "slimy" colonies, does not liquefy gelatin (culture assumes a characteristic nail-like appearance).

All sugars, except lactose are fermented with the formation of gas.

CHAPTER XVII

THE TETANUS BACILLUS (LOCKJAW)

I. Historical

The bacillus tetani was discovered by Nicolaier in 1885 and first studied and cultured by Kitasato in 1888.

II. Morphology

The bacillus is a slender organism, possesses spores, no capsules; the vegetative forms are motile and have flagella. Spores are developed after twenty-four to forty-eight hours of incubation. The spore-bearing forms resemble a drumstick, since the spore is usually at the end of the bacillus. (See Fig. 4.)

The bacillus is Gram-positive.

III. Cultural Characteristics

Bacillus tetani is a strict anaerobe; it grows readily on meat infusion media. Gelatin is slowly liquefied, milk is not coagulated.

IV. Destruction

The vegetative (non-spore-bearing) form are readily destroyed by ordinary disinfectants and heat, but the spore-bearing forms resist dry heat at 80° C. for one hour and even live steam for five minutes, and it takes twelve to fifteen hours for a 5 per cent solution of carbolic acid to kill them.

Protected from sunlight the tetanus spores will live for years.

V. Disease Production, Mode of Infection, Disinfection and Prophylaxis

The tetanus bacilli are found in superficial layers of soil, especially near stables and manured fields.

Bacillus tetani produce a disease called lockjaw, characterized by convulsions and closing of the jaws. Deep lacerated wounds where there is much tissue destruction, as, for example, gun-shot wounds, are especially favorable for the development of the tetanus.

The true prophylaxis consists in administering antitetanic serum in all gunshot wounds, or where the patient had stepped on a nail, especially near the tilled soil.

VI. Mechanism of Infection and Immunity

The tetanus bacillus produces an exotoxin, which has a special affinity for the nervous system. It is one of the most poisonous substances known, one-millionth of a gram being sufficient to kill a mouse.

The common fowl is extremely resistant to tetanus, while the man and horse are unusually susceptible.

The animal injected with nonfatal doses of tetanus produces an antitoxin as the only antibody.

VII. Bacteriologic Diagnosis

Bacteriologic diagnosis is easy and consists in finding a Gram-positive, "drumstick" like spore-bearing bacillus; culturally it is an anaerobe, growing well on meat infusion media.

VIII. Immune Treatment

Immune treatment is both prophylactic and curative, and consists in administering the antitetanic serum, intravenously or intraspinally. The serum is prepared by injecting a horse with gradually increasing doses of the tetanus bacilli or broth filtrate, and obtaining the horse's blood, separating the blood serum and testing its antitoxic (that is, exotoxin-neutralizing properties).

IX. Summary of Important Characteristics

B. tetani causes lockjaw, is a Gram-positive, spore-bearing, motile (in spore form) bacillus, having the appearance of a drumstick, anaerobe, produces exotoxin; antitetanic serum is a perfect preventive and a good curative agent, if not given too late.

CHAPTER XVIII

BACILLUS OF SYMPTOMATIC ANTHRAX. THE ANTHRAX BACILLUS. BACILLUS AEROGENES CAPSULATUS. BACILLUS OF MALIGNANT EDEMA

Bacillus of Symptomatic Anthrax

But a few lines are sufficient for the description of this organism, as it never causes disease in man, who appears to be quite immune to this infection.

The symptomatic anthrax has nothing whatever to do with anthrax (to which man is not immune). It is a disease of cattle, sheep and goats (the disease is also called "quarter evil" and "blackleg"). Like tetanus bacillus the bacillus of symptomatic anthrax is a spore-bearing, strictly anaerobic organism; the vegetative forms are motile and have numerous flagella. It is Gram-negative. It grows well on simple media. When infection takes place there is a soft, puffy, creaking, swelling, rapidly spreading, death following in three to ten days. It produces an exotoxin.

The Anthrax Bacillus

Anthrax occurs both in animal and man. It is an ancient disease well known from the earliest days of the history, and is especially common in Russia. Historically it is of especial interest as it was the first organism proved to be a specific cause of an infectious disease.

It was discovered by Pollender in 1849, and thoroughly studied by Davaive in 1863. (See Fig. 31.)

The bacillus is a very long rod, 5 to 10 μ long. It occurs singly in blood, but grown on artificial cultures, is found in long threads. It has spores and is Gram-positive. A capsule is seen occasionally (never when grown on artificial media). The anthrax is an aerobic organism, facultatively anaerobic, and grows well on ordinary culture media. It is nonmotile, has no flagella. Spore formation ceases below 18° C. and above 42° C.

Fig. 30.—Bacillus welchii. Film preparation of a milk culture. x. 1000. (Hewlett—*Manual of Bacteriology.*)

Because of its spores the anthrax bacillus is very resistant to heat and ordinary chemicals, it takes 10 minutes' exposure to live steam to kill them.

In man three varieties of infection are produced: (1) skin ("the wool sorters' disease"), (2) lungs, and (3) gastrointestinal. No other disease shows so many bacilli in blood as the anthrax infections. It produces an endotoxin. Pasteur's vaccine is a wonderfully efficient treatment of the infection in the cattle (the cultures for the

vaccines are grown at 42° to 43° C., so that no spore-forming bacteria are produced).

Bacillus Aerogenes Capsulatus

Bacillus aerogenes capsulatus was discovered by Welch of Johns Hopkins University, in 1892.

The organism is 3 to 6 μ long, usually occurs single, has spores, is nonmotile, has no flagella, has a capsule, is Gram-positive.

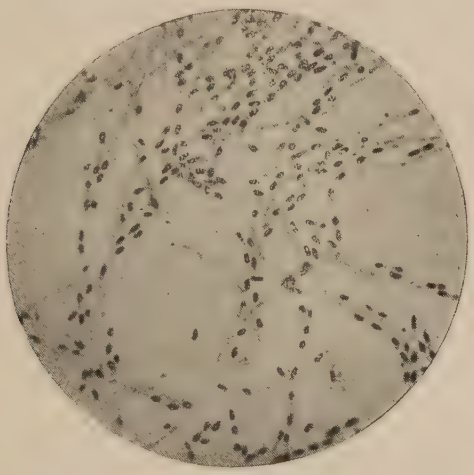


Fig. 31.—The anthrax bacilli showing spores. (Mallory and Wright—*Pathological Technic.*)

This bacillus is a strict anaerobe and grows on all simple media. Gelatin is slowly liquefied. Grape sugar, milk and cane sugar are all fermented.

The infection usually occurs after injury, especially after open fractures. In the present war this infection has been of great importance, and it seems that gunshot wounds offer great opportunity for such infections, es-

pecially where there is well manured soil. Spores have been repeatedly found on the uniforms of soldiers.

A new ray of hope is offered by the brilliant work of our own bacteriologist, Bull, of the Rockefeller Institute, who has succeeded in preparing an antitoxin (the organism producing an exotoxin) which has proved highly successful in animal experimentation, and which Bull is now trying out on patients on the battle fields.

Bacillus of Malignant Edema

The bacillus of malignant edema was discovered by Pasteur in 1877.

This is a slender long rod, motile, with numerous flagella, has spores, and is Gram-negative. It is a strict anaerobe, grows readily on simple media. It produces acute inflammation and (edema) swelling which extends deeply into the tissues, and is very dangerous.

CHAPTER XIX

BACILLUS BOTULINUS. BACILLUS MALLEI. BACILLUS PYOCYANEUS

Bacillus Botulinus

Bacillus botulinus causes about 25 per cent of all meat poisoning, a very dangerous disease. It was discovered by Van Ermengem in 1896. It is a large rod, 4 to 6 μ, motile, has flagella, no spores, no capsules, is Gram-positive, is a strict anaerobe, grows well on meat infusion media. It produces an exotoxin.

Bacillus Mallei

Bacillus mallei causes glanders. It is a small bacillus, is nonmotile, has no flagella, no spores, no capsules, is Gram-negative, stains with methylene blue, it is not unlike the diphtheria bacillus, stained parts alternate with faint parts, grows well on meat infusion media.

It produces an endotoxin.

In diagnosing the disease, the mallein test is used: a small amount of the endotoxin is injected in the skin, and the presence of infection, a severe reaction takes place.

Bacillus Pyocyaneus

Bacillus pyocyaneus causes green suppurations which are fortunately rare, for while they are not dangerous, they seriously interfere with healing. Occasionally kidney infections are caused by this organism (the author saw three cases within six months).

The organism was discovered by Gessard in 1882. It is a short rod, Gram-negative, motile, has flagella, no spores, no capsules. It is an aerobic organism, facultatively anaerobic, grows well on simple media on which within twenty-four hours, a green pigment is formed. It produces both an exotoxin and endotoxin. As to the treatment a new method has been evolved during the present war which is by far better than anything so far used; this consists of applying a 1 per cent solution of acetic acid to infected areas; in three cases treated by this method at the Charity Hospital all three cases showed complete disappearance of green discharge within twenty-four to forty-eight hours.

CHAPTER XX

THE DIPHTHERIA GROUP

I. Historical

Klebs in 1883 and Loeffler in 1884 were first to discover and describe the organism.

II. Morphology

The organisms are slightly curved rods, 4 to 5 μ, they are not uniform in thickness, usually being club-shaped. The best stain for the diphtheria bacilli is Loeffler's "alkaline methylene blue," the bacilli show a characteristic lack of uniformity in staining so that the stained preparations show lighter parts alternating with darker, shaded parts, the bacilli possessing a characteristic "beaded" appearance. It is a Gram-positive organism. It has no spores, capsules, or flagella.

III. Cultural Characteristics

The organism is an aerobic one, growing best at 37.5° C., although it may grow between 20° C., and 42° C. The best medium to grow the bacilli in, is Loeffler's beef blood serum (see Chapter on The Culture Media Making). On this medium the organism show small, grayish white, glistening colonies.

IV. Resistance

The organism is killed by ten minutes' exposure to 58° C. Low temperatures are well borne, and it may

resist drying for several weeks. Chemical disinfectants easily destroy the organism.

V. Disease Production, Mode of Infection and Prophylaxis

Diphtheria is usually confined to the throat, larynx, and nose, but it may attack other mucous membranes for which the bacillus seems to have a special predilection.

Diphtheria is transmitted by coming in contact with those suffering with disease. For this reason all such persons should immediately receive an injection of diphtheria antitoxin; the patient should be isolated, quarantined, and the strictest precautions should be taken in

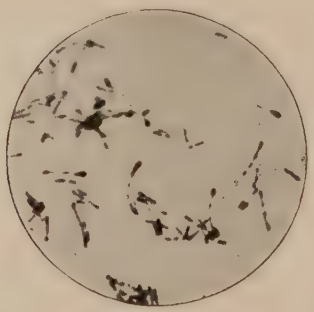


Fig. 32.—One of the very characteristic forms of diphtheria bacilli from blood-serum cultures, showing clubbed ends and irregular stain. x 1100 diameters. Stain, methylene blue. (Park and Williams—*Pathogenic Bacteria and Protozoa.*)

disinfecting his or her utensils, linen, etc. Severe epidemics are frequent, usually in institutions, such as schools, asylums, etc.

A very important question today is that of diphtheria bacilli carriers. It is known that many persons may harbor the diphtheria bacilli in their throats and yet show no signs of the disease; in spite of this, however, they are always a source of danger to others; and therefore,

the patient should not be allowed to mingle with others until at least two cultures made from their throats have been found negative.

The Schick test is based on the principle that persons whose natural content of antitoxin is low, i. e., insufficient to protect them from diphtheria, can be identified by injecting into their skin a small amount of diphtheria toxin when in such persons a marked reaction will take place at the site of injection, while in persons whose content of antitoxin is normal, no such reaction will take place. In practice the amount of diphtheria toxin injected is 1/50 of the amount sufficient to kill, in four days, a guinea pig weighing 250 grams.

Prevention of diphtheria means isolation and quarantine of the patient and nurse, strictest disinfection of all utensils, bedding, etc., and injection of diphtheria antitoxin to all those who had come in contact with the patient. Schick's test will show who needs such injections.

VI. Mechanism of Infection and Immunity

Diphtheria bacillus, like the tetanus, produces an exotoxin; it was in connection with this exotoxin that most of the experimental work on immunity was done by Ehrlich and his pupils. The antibody produced in infected animals is, of course, an antitoxin. The antitoxin is produced on a large scale, by injecting horses with gradually increasing doses of toxin, until according to Weigert's law, many more antibodies (antitoxin) are produced than are really necessary to protect the horse. The blood serum is removed, standardized (that is, its strength is determined), and it is then ready for use. It is evident that immunity conferred by the injection of antitoxin serum is acquired artificial, passive immunity.

VIII. Bacteriologic Diagnosis

Bacteriologic diagnosis rests on peculiar beaded appearance of a bacillus, which is often club-shaped, Gram-negative; cultural appearance on Loeffler's beef blood serum is diagnostic: small, round, glistening, grayish white colonies.

VIII. Immunity Treatment

Together with the smallpox vaccination, the prevention and treatment of diphtheria with the antitoxin serum (initiated by Behring, Roux and Yersin) is, without

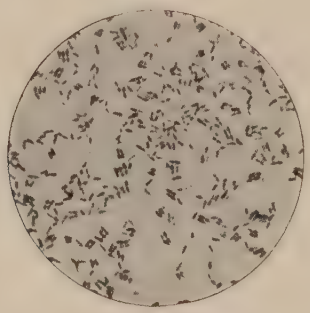


Fig. 33.—Pseudodiphtheria bacilli. (B. hofmanni.) (Park and Williams—*Pathogenic Bacteria and Protozoa.*)

doubt, the greatest accomplishment achieved by medicine. No one today realizes what diphtheria meant in former days: death was inevitable; during an epidemic, mourning crepe would be hung out on one house after another, an eloquent and silent witness of the dreadful scourge. Today a death from diphtheria is a scarlet spot on a physician's escutcheon, there is absolutely no excuse for death from diphtheria today, for, unlike the tetanus antitoxin serum which is a much better prevention than a cure, the diphtheria serum is an extremely efficient cure,

as well as preventive; the earlier the administration of the serum is begun the better the results.

IX. Summary of Important Characteristics

A slender Gram-positive bacillus, usually club-shaped, presenting when stained, a beaded appearance, growing in beef blood serum in small, glistening, grayish colonies, nonmotile, causes diphtheria; possesses an affinity for mucous membranes, produces an exotoxin, the body reacting by the production of antitoxin. Prevented and cured by the antitoxin serum. The pseudodiphtheria bacillus (Hoffmann's bacillus) is shorter and thicker, the beads or bands in stained preparations seldom exceed two or three. It grows more abundantly and colonies are whiter than those of the diphtheria bacilli.

CHAPTER XXI

THE TUBERCULOSIS GROUP

Tuberculosis, or consumption, is an infection which occurs most often in the lungs, but no organ is safe; the bones, lymph glands, intestine, peritoneum, genital organs, skin, all frequently fall victims of the "white plague."

I. Historical

Koch, in 1882, was first to isolate and cultivate the bacillus.

II. Morphology

The bacillus is a slender rod, 2 to 4 μ in length. They are nonmotile, have no flagella, no spores, no capsules, but are said to have a cell membrane which enables them to resist drying. It does not stain with ordinary dyes, and a special method is used for the purpose (see chapter on Staining in General Bacteriology); because, once stained, it resists the action of strong acids, the tubercle bacillus is called "acid-fast." It is Gram-positive.

III. Cultural Characteristics

The tubercle bacillus is very difficult to cultivate. Special media (described in chapter on Culture Media) are necessary for successful cultivation. Growth takes place from the tenth day on; when well developed the growth has a characteristic "bread crumb" wrinkled dry

appearance. The tubercle bacillus is an aerobic organism.

IV. Destruction

Dry heat (100° C.) will be resisted for one hour. The tubercle bacilli are resistant to cold and very resistant to drying; in sputum the bacillus remains alive for two or three months. A 5 per cent carbolic acid solution kills the bacilli in a few minutes, sunlight kills them in a few hours.

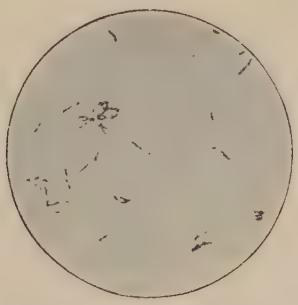


Fig. 34.—Tubercle bacilli, human. x 1000 diameters. (Park and Williams—*Pathogenic Bacteria and Protozoa.*)

V. Disease Production, Mode of Infection, Disinfection and Prophylaxis

Disease is produced both by inhalation (of infected dust) and ingestion (of infected meat, milk, etc.).

Formation of small nodules, the so-called "tubercle," is the characteristic feature of the disease; this may be followed by ulceration and cavity formation. Unsanitary environment is the best ally the disease has; crowded, dark rooms, lack of fresh air, sunlight, nourishing food—is the favorite playground of tuberculosis. It is not a hereditary disease; the reason that so many children of tuberculous parents develop the disease is

not that they were born of tuberculous parents, but because they live with them. Prevention means isolation of the patient, disinfection of sputum and other excreta and linen, etc., fumigation of the room, etc. See special chapter on Disinfection and Fumigation.

VI. Mechanism of Infection and Immunity

The tubercle bacillus produces an endotoxin (tuberculin). Tuberculin, prepared in several ways, is used for diagnosis of the infection; no cattle is to be killed for food unless it has been tested with tuberculin, as also should all cows producing milk for sale. In human beings the tuberculin test is applied into or under the skin, and in infected individuals. Such injection causes a marked reaction at the site of injection. This test is called von Pirquet's test. The antibodies produced by infected animals and human beings are essentially bacteriolysins.

VII. Bacteriologic Diagnosis

Bacteriologic diagnosis rests on finding the bacilli stained by the special method (described in chapter on Staining). If this fails, sputum or urine may be injected intraperitoneally into a guinea pig and if the animal does not die in three or four weeks, it is killed, and evidences of tuberculosis are looked for at the site of injection, in the lungs, liver and spleen. Tuberculin test and, recently, complement-fixation test (similar to Wassermann test for syphilis) are of great value.

VIII. Immune Treatment.

No such treatment is very successful, although various authors have claimed good results obtained in some cases treated with small doses of tuberculin.

IX. Summary of Important Characteristics

A slender rod, stained by special method, resists drying and heating better than most of the bacteria, requires special methods of cultivation. Tuberculosis is the most widely spread disease, not only among human beings, but also among cattle (bovine tuberculosis), birds (avian tuberculosis), fish, etc. Any organ in the body may be involved, but the lungs are the most frequently affected organ. Very contagious disease, favored by unhygienic surroundings, requires strictest disinfection; diagnosis rests on finding the bacilli in sputum, urine, tissue, or on animal inoculation, tuberculin test and complement-fixation test. Produces an endotoxin, no immunity treatment is successful.

CHAPTER XXII

THE BACILLUS OF LEPROSY. THE SMEGMA BACILLUS

The Bacillus of Leprosy

Bacillus lepræ is the organism that causes the horrible, incurable disease called leprosy. It resembles the tubercle bacillus very closely except that it is not quite as

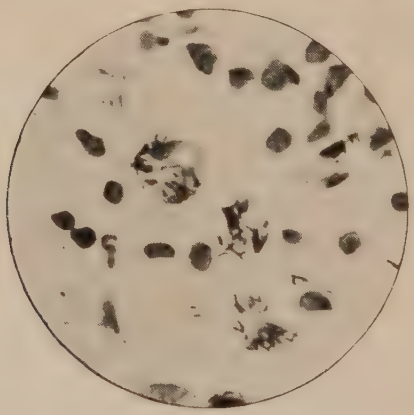


Fig. 35.—Leprosy. Section of skin. x 1500. (Hewlett—*Manual of Bacteriology.*)

slender. The disease is almost never met with in this country, and the bacteriology of the affection is, therefore, unimportant. The disease is less contagious than most other bacterial diseases, and those who observe ordinary precautions of cleanliness seldom, if ever, contract it. The differentiation between the tubercle and leprosy bacilli is practically impossible.

The Smegma Bacillus

The smegma bacillus is a harmless (saprophytic) organism, which occurs around the genital organs of both male and female, and is important because it may be readily confused with the tubercle bacilli. The best method of differentiation between the two is that of Pappenheim.

Fig. 36.—The smegma bacillus. Smear preparation of smegma. x 1500. (Hewlett—*Manual of Bacteriology.*)

1. Make smears and fix by heat in the usual manner.
2. Stain with Zeihl's carbol-fuchsin solution (steaming) for two minutes.
3. Pour off the stain, and without washing, pour the following mixture:
4. Wash in water. Smegma bacilli will be decolorized, but the tubercle bacilli will be stained bright red.

Rosolic acid .....1 gram
Absolute alcohol .....100°C.
Methylene blue added to saturation.
Glycerine (added after other ingredients have been mixed).....20°C.
This mixture is poured on and off several times.

CHAPTER XXIII

THE INFLUENZA GROUP. THE BACILLUS PERTUSSIS. BACILLUS PESTIS. THE CHOLERA GROUP. THE BACILLUS OF DUCREY

The Influenza Group

Influenza (la grippe, grip) is the most contagious disease known, and it spreads over large territories with unbelievable speed. In 1889 and 1900, it traversed the entire world. The bacillus was discovered by Pfeiffer in 1892.

It is an extremely small organism, 0.5 μ long, it is Gram-negative, nonmotile, has no flagella, no spores or capsules. It grows with difficulty, the best medium being the meat infusion agar with the addition of whole blood. It is an aerobic organism, is readily destroyed by heating, drying, and ordinary disinfectants.

It is extremely difficult to guard against the contagiousness of this disease, and the patient should be isolated. It produces an endotoxin.

The Bacillus Pertussis

The bacillus pertussis causes whooping cough, and was discovered by Bordet and Gengou in 1900. It is a very small, short bacillus, almost resembling a coccus, Gram-negative, nonmotile, has no flagella, no spores or capsules. It grows on defibrinated blood agar potato. Patients with whooping cough should be isolated. The complement-fixation test is used in the diagnosis, but is

not yet perfected; the bacillus produces an endotoxin. The immunity treatment—the pertussis vaccine—is of great value in shortening the disease and hastening recovery.

Bacillus Pestis

Bacillus pestis which causes the most fatal of all diseases—the bubonic plague—was discovered by Kitasato

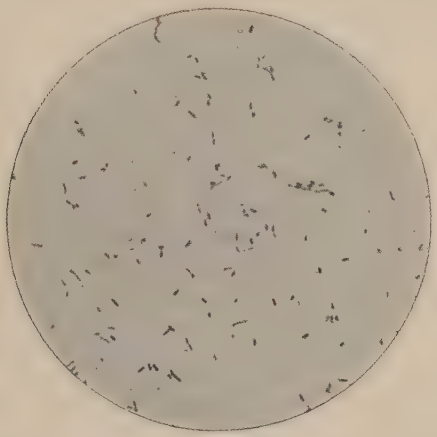


Fig. 37.—Influenza bacilli. x 1100 diameters. (Park and Williams—*Pathogenic Bacteria and Protozoa.*)

and Yersin in 1894. It is difficult to grasp the havoc wrought by this scourge; in the reign of Justinian one-half of the population was wiped out by the plague. Known as "Black Death," it swept the entire world in the fourteenth century, killing 25,000,000 people. It is always present in India exacting as its toll several thousand lives yearly. Bacillus pestis is a thick, short bacillus, Gram-negative, has no spores, no capsules, no flagella, and is not motile. It grows well on meat infusion media. It is aerobic, and may remain alive for months and years

in the dark, if sufficient moisture is present. It lives for two weeks in the pus and sputum of patients. Complete drying kills the bacilli in two to three days, live steam in a few minutes. The disease is usually acquired by entrance of bacilli either through the skin or by the respiratory tract, in the latter case the disease is not unlike pneumonia. The disease is extremely dangerous causing great mortality.

The bacilli are usually transmitted by rats and ground

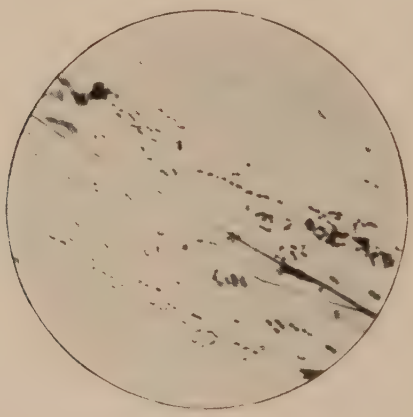


Fig. 38.—Bacillus pestis. Smear preparation of sputum. x 1000. (Hewlett—*Manual of Bacteriology.*)

squirrels. One attack of plague usually confers immunity. Both the vaccine and the serum treatments have been used for prevention with encouraging results.

The Cholera Group

Cholera, which at times becomes epidemic and invades large territories, is but little known in this country. It is a very dangerous disease, characterized by persistent diarrhea and collapse. The "comma bacillus," so-called

because of its shape, or the spirillum cholera was discovered by Koch in 1883. It is a small curved rod, actively motile, has one flagellum, no spores or capsules, is Gram-negative and grows readily on meat extract media. It is an aerobic organism, and can be quickly killed by ordinary antiseptics. The disease is contracted by eating contaminated food or drinking impure water. It is essentially a "water-borne" disease, as has been shown in the famous epidemic of Hamburg (1892) where

Fig. 39.—Spirillum choleræ. Film preparation of a pure culture. x 1500. (Hewlett—*Manual of Bacteriology.*)

a terrible epidemic of the disease raged, while the town of Altona (as close to Hamburg as Brooklyn is to New York) which had a separate water supply, was scarcely touched. The prevention during an epidemic means a thorough filtration of water or the use of boiled water and the patient should be handled in the same manner as a typhoid case.

The cholera spirillum produces an exotoxin and the infected animal elaborates bacteriolysins; it was in connection with the cholera spirillum that Pfeiffer has

demonstrated bacteriolysins. One attack usually confers a permanent immunity. No successful immune treatment has as yet been developed.

The Bacillus of Ducrey

Bacillus of Ducrey is the organism which produces chancroid, an acute inflammatory lesion on the genital organs. It is acquired by direct contact. The bacillus is very small, nonmotile, has no flagella, no spores, no capsules. It is Gram-negative and grows on blood agar.

CHAPTER XXIV

THE SPIROCHETAL DISEASES

Spirochetes or treponemata belong to a class which is half way between the true bacteria and protozoa (the lowest order of animal). They are corkscrew-like, wavy threads. Several very important diseases are caused by the various spirochetes. (See Fig. 40.)

I. Syphilis

Syphilis, probably, is the most disseminated disease, transmitted by direct contact, sexual or asexual, caused by Spirocheta pallida, discovered by Schaudinn and Hoffmann in 1905. It must be stained by special method, not to be attempted by the beginner, and can be grown anaerobically on ascitic fluid agar to which a piece of sterile rabbit kidney has been added. Noguchi, of the Rockefeller Institute, is the discoverer of this method.

One of the simplest methods of staining is to place a drop of material from a syphilitic lesion on a glass slide and mix it with a drop of India ink, then make a smear as if a blood smear is to be made. Examined with the oil-immersion lens the spirochetes appear unstained against a black background.

Within a year of the discovery of the Spirocheta pallida, Wassermann, Neisser and Bruck devised a special test known as the "Wassermann test," which shows the presence of the disease, even though no symptoms or signs are detected.

A year later Ehrlich discovered the famous Salvarsan (606) which, combined with mercury, insures the cure in practically all cases. Truly, this has been a wonderful age!

Syphilis may be transmitted from the parents to the offspring.

Fig. 40.—Various types of spirochetæ. (MacNeal—*Pathogenic Organisms.*)

II. Relapsing Fever

Relapsing fever, rarely seen in this country, is caused by Obermeier's spirochete (1873). This is a much longer organism than that of syphilis. It can be cultivated by Noguchi's method, like the Spirocheta pallida of syphilis. The disease consists of recurrent attacks of fever.

III. Vincent's Angina

Vincent's angina, a variety of "sore throat" is caused by Vincent's spirillum, which is a small spirochete, the disease is a mild one and yields readily to local treatment.

IV. Yaws, or Frambesia

Yaws, or frambesia, is a disease which occurs in tropical countries and resembles syphilis. It is caused by Spirochete pertenuis.

CHAPTER XXV

MALARIA. THE TYPHUS FEVER

Malaria

The organism causing malaria was discovered by Laveran in 1880. There are three forms of malaria: tertian, when chills recur every forty-eight hours; quartan, when they recur every seventy-two hours; and æstivo-autumnal—more or less irregular. Each of these varieties is caused by a different parasite, the tertian by plasmodium vivax, the quartan by plasmodium malariæ, and the æstivo-autumnal by plasmodium falciparum. The æstivo-autumnal fever is the most dangerous of all forms, but is seldom met with in the temperate climate. The parasites are transmitted by mosquitoes, whose bite carries them into the human body where they pass through regular phases of development within the red blood cells, multiplying and breaking into numerous young forms, which are liberated into other red blood cells. When a mosquito bites a malarial patient he carries these young forms, which undergo the second cycle (sexual) of development within the stomach of the mosquito, then new forms reach the salivary glands of the mosquito and are transmitted to human beings with the bite.

In order to demonstrate the malarial parasite in the human blood, a blood smear is made on a glass slide or a cover-slip and is stained with Wright's stain just as an ordinary blood preparation is made. The appearance of the parasites vary according to the variety and the stage

of the development. The prevention of malaria requires the destruction of swamps and marshes—the breeding places of mosquitos, the screening of the houses and the administration of quinine, which is a specific for malaria.

The Typhus Fever

Typhus fever, which has nothing to do with typhoid fever, is very seldom met with in this country, but is very frequent in some parts of Europe—Russia, Roumania, Servia, etc. The disease is characterized by severe fever, rash and intoxication symptoms, and is a very dangerous malady. The organism causing it was discovered in 1914, by an American bacteriologist, Plotz. It is an anaerobic bacillus, and has been definitely proved to be the true cause of typhus fever. No definite work has yet been completed with regard to immune treatment, but it is well known that the prevention depends on cleanliness, as the bacilli very often are carried by body lice.

CHAPTER XXVI

THE HIGHER BACTERIA. THE YEASTS. THE MOLDS

The Higher Bacteria

The higher bacteria are of more complex structure than the ordinary bacteria and cause a number of diseases.

I. Streptothrix

The streptothrix causes an infection of skin and, occasionally, of other organs. It usually appears in stained preparations (with Loeffler's alkaline methylene blue) as rods and branching thread-like filaments.

Streptothrix can be cultivated on sterile fresh kidney tissue of rabbits.

II. Actinomycosis

Actinomycosis is the "lumpy jaw" of the cattle, and not infrequently seen in human beings, in whom it affects not only the jaw, but the skin, lungs and the intestinal tract as well. In pus the parasites appear as small granular bodies, grayish or yellowish. Microscopically they appear as rosette-like matter with the characteristic, club-shaped bodies.

The Yeasts

Of these, several cause disease in man, especially the so-called Saccharomyces hominis. Another yeast infec-

tion is the disease of the skin called blastomycosis and sporotrichosis.

The Molds

The molds cause a number of skin diseases and a disease of the mouth (most common in children) known as thrush. Of the skin diseases caused by molds the common ones are ringworm, favus, pityriasis versicolor, etc.

SECTION III

CHAPTER XXVII

DISEASES OF UNKNOWN CAUSATION

Smallpox

Smallpox, the most virulent of all infectious diseases, used to be a veritable scourge, but today is practically subdued, thanks to the epoch-marking discovery of Jenner, who, in 1718, introduced vaccination for smallpox and thus conferred an everlasting obligation upon mankind. The vaccine is prepared from the infectious material taken from the calves which have been inoculated with the material from human smallpox.

Measles

Measles is essentially a disease of childhood. Nothing definite is known about the organism causing it.

Mumps

No specific organism has been discovered for mumps.

Scarlet Fever

Scarlet fever is also essentially a disease of childhood. Several investigations have claimed to have discovered the specific organism, the last one being Mallory, of Boston, but so far no definite conclusions have been drawn.

Trachoma

Trachoma, a dangerous disease of the lining of the eye (conjunctiva), is very common among the Indians and the poorer classes of some European countries. No specific organism has been discovered.

In all these diseases strictest isolation and quarantine is necessary, as well as strict disinfection and fumigation.

Infantile Paralysis

Infantile paralysis is called "acute anterior poliomyelitis. It is an infectious disease which has become a very serious menace, as some alarming epidemics have occurred within the last ten years.

No definite organism has as yet been discovered, but some very promising work has been, and is being, done by Flexner, Rosenow, and others, and the near future will probably see the solution of this disease.

Yellow Fever

Yellow fever is an acute infectious disease, rare in this country, but prevalent in the tropics. It is transmitted by the variety of mosquito called Stegomyia fasciata; the organism causing it is unknown. The bulk of the work on yellow fever is a glorious chapter in American bacteriology. Reed, Carrol, Lazear, all of the United States Army, have solved the mystery of the transmission of yellow fever, all three having sacrificed themselves to the altar of science. The brilliant work of eradicating yellow fever in Panama is due to the present Surgeon-General of the United States Army, Gorgas, and is probably one of the most splendid prophylactic achievements in the world's history.

Dengue

Dengue is an acute infectious disease characterized by chills, fever, bone ache, etc.; the organism causing it is unknown.

Rocky Mountain Spotted Fever

Rocky Mountain spotted fever, a disease propagated by tick bites, is restricted to the region of the Rockies. The organism is unknown.

Foot and Mouth Disease

Foot and mouth disease occurs chiefly in cattle, sheep, and goats. It appears as a blistery eruption in the mouth and on the skin between the hoofs. The specific organism causing it is unknown.

Hydrophobia, or Rabies

Hydrophobia is a fatal infection of practically all mammalia. Although the organism is unknown, Pasteur's brilliant work has given us an absolute cure against it, if applied early.

The infectious agent is transmitted in the saliva of the mad animal. By special staining methods (the best is that of Lentz) we can demonstrate in the brain of mad animals the so-called "Negri bodies," which are characteristic of the disease.

The Pasteur treatment consists of administering a vaccine prepared from the cord of rabbits inoculated with the spinal infectious material. It absolutely prevents the disease if administered early. Whenever one is bit by an animal (usually a dog), the latter should be killed and its brain should be examined; the Pasteur treatment should be at once instituted.

CHAPTER XXVIII

BACTERIA IN SOIL, AIR, WATER, AND MILK

The nurse who can give relief and comfort to those already diseased, and at the same time can protect herself and others from becoming infected, is in a true sense, capable. To do this, it is important that she not only have a knowledge of bacteria after they have gained access to the body, but also that she be informed as to the means by which these organisms live and are detected in other environments.

The surroundings that are the most vital in sustaining life in the human body, and are often the haven for harmful bacteria are soil, water, air, and milk.

Microorganisms of the Soil

The number and kind of bacteria found in the soil are relative, depending largely upon its fertility, climatic conditions, and its relation to plant and animal life; as we are interested only in the pathogenic microorganisms we can exclude the great list of bacteria and molds, which, while not important in this study, are absolutely indispensable in plant life.

Pathogenic bacteria found in the soil, those giving the most trouble are: the B. welchii (gas bacilli), tetanus (lockjaw), typhoid bacilli, and spirilla of Asiatic cholera. The first two apparently thrive in the soil, the latter, unless under very favorable conditions, lie dormant and, in a short time, disappear. It is questionable if any pathogenic bacteria find the soil their natural habitat

or rather, if they are not carried there by dead organic matter and excrement. For full detail of these organisms, see special chapter given to each.

Microorganisms of the Water

Nothing is more important to a community than that it is supplied with pure water. Not only that it is clear and palatable, but it must be free from pathogenic bacilli. The eye is no criterion in this matter, for water that may look clear and pure will often contain many harmful bacteria, while cloudiness and odors may be due to recent disturbances, to harmless plants, etc.

Most waters contain many bacteria, but the presence of pathogenic bacteria is due to pollution either by sewerage or imperfect drainage. Where the water supply is obtained from a well, great care must be taken in selecting a site that is free from the drainage of stables, privy vaults, etc. In the city where the source of supply is from lakes and rivers, constant watch must be kept to see that the sewage and industrial wastes do not render it unfit for use. Pools and swamps are often a menace to public health, for while this water may not be taken into the body for drink, they furnish a breeding place for insects that often carry disease germs.

A large body of water tends to be self-purifying. When contamination occurs by the natural processes, i. e., sedimentation, oxidation, and the continual action of sunlight, these harmful bodies are destroyed. Sedimentation is the gravitating of the particles to the bottom, carrying with them impurities and bacteria. The action of oxygen and sun rays destroys many bacteria, especially those on the surface.

Water-borne Disease.—When pollution occurs, the disease germs that are usually found are the typhoid-colon

group, dysentery and cholera. Most other harmful bacteria are short-lived in water, but those mentioned thrive there, and have often caused severe epidemics.

Should water become contaminated with these germs, it may be purified by boiling and distillation. Where a large body of water must be purified, science has devised the filters and the action of chemicals. With the use of these agents from 95 to 98 per cent of the bacteria is destroyed.

Many cities now filter their water supply. The water is pooled in large reservoirs. The bottom of these reservoirs is composed of a layer of coarse and a layer of fine gravel, on top of this is a strata of coarse and fine sand. The water percolates through these at a certain rate, as it does so the impurities and bacteria are deposited. After a certain time the deposit must be removed.

There are many kinds of domestic filters, although only a few can be recommended for permanent use. Many, particularly the cheaper kind, are actually harmful. It is impossible to clean them properly, so that the bacteria they hold back breed there. The better and more expensive types, to be efficient, need constant care and should be cleaned and sterilized at regular intervals.

When the germ content of water is unknown, during certain seasons and epidemics, the use of boiled water for drinking purposes should be encouraged. All bacteria are destroyed by this process. The flat, insipid taste resulting may be partially overcome by shaking the water in open vessels, aerating it.

Distillation is the process of driving steam into a cool jar where it condenses; the water resulting is not only free from bacteria but all mineral. It is used largely in the laboratory, but is not recommended for drinking purposes. When taken into the body in large quanti-

ties, by osomosis it tends to extract the salts and minerals from the tissues.

Most communities have a regular bacteriologic examination made of the water supply; knowing the number of bacteria present in the water under normal conditions, any deviation from this standard must mean danger.

A sample of water to be tested may be taken from the faucet, first allowing the water to run for thirty minutes. When it is not possible to obtain a specimen in this way it should be collected in sterile vessels, kept at a cool, even temperature and hastened to the laboratory.

The number of bacteria in the water is determined by placing 1 c.c. of the sample in a sterile Petri dish, add to it a tubeful of melted agar or gelatin, mix and let stand for three or four days in a dark, moist atmosphere, at 37° C. If the number of colonies are small, they may be counted very easily. The result may be taken as the number of individual bacteria contained in a quantity of water measured. If the number of colonies are large the original specimen of water must be diluted with sterile water or a large number of colonies may be counted with the aid of some mechanical device. The Wolffhuegel plate is generally used, although any black surface ruled in squares with white lines may be used. A certain number of these are counted, then the average is obtained.

The typhoid and cholera bacilli are difficult to isolate from contaminated water, or rather it is difficult to differentiate the typhoid from the colon bacillus, unless cultural tests are made (see chapter on Typhoid-Colon Group). The presence of colon bacilli usually indicates the close proximity of sewage pollution.

Bacteria of the Air

In the early days of surgery, it was thought that infection was caused mainly through the air. Working upon this principle antiseptic sprays were used. Later sterilizing instruments, gloves, clothing, etc., and keeping the air free from dust as much as possible was instituted. Sprays were then discontinued.

All infection does not come through the air, yet it is the means of spreading some diseases. Microorganisms being so small and light are by any draft or sweeping, disturbed, and suspended in the air. In coming to rest, if they chance upon a suitable media, they thrive, and infection occurs.

The air in a crowded room is filled with bacteria, likewise the air in the open contains more organisms on a dry, windy day, than in moist weather. To lessen contamination from the air, laboratories and operating room should be free from drafts. As an added precaution, to prevent the exhaled breath from blowing organisms in the air, many surgeons breathe through gauze mouthpieces. When bacteria are found in the air they usually exist as spores and molds. Living tubercle bacilli have been found in the air after sweeping the room where tuberculous patients live. Anthrax bacilli have been isolated from the air which surrounds the stable of an animal suffering from that disease.

Smallpox, measles, influenza, scarlet fever, and other diseases of unknown origin are sometimes called air-borne diseases. This term should not be taken literally, as "water-borne" diseases is, for the organisms causing these illnesses do not live in the air, but are only suspended or carried by it. They are picked up from dry sputum or pustules, or sent in the air by sneezing or coughing.

Sedgwick-Tusher's aerobioscope is the most accurate instrument used in studying organisms of the air. A fair result may be obtained by exposing a Petri dish containing culture media. Opening it for one to two minutes should be sufficient. It would be interesting to do this before and after sweeping a room.

Bacteria of Milk

Milk, having great nourishing qualities, is one of our chief articles of food. On account of its great food value it furnishes a favorable media for the growth of bacteria.

By careful handling, the bacteria count of milk may be kept down. This is accomplished by submitting the cows to the tuberculin test, to see that they are free from disease, by sanitary stables free from flies and dust, by clean utensils and milkers, and keeping the milk cool until consumed. On account of the expense, such methods are not always employed, and a form of sterilization is used. Pasteurization is generally used to keep milk pure. The milk is heated in a water-bath to 160° F., for twenty minutes. There are several domestic pasteurizers. They are especially useful in infant feeding. Boiling for ten minutes is another method for destroying the bacteria, but as boiled milk has a rather unpleasant odor and taste, it is not generally used.

Pathogenic organisms in milk usually come from unhealthy cows, infecting it with tuberculosis, foot and mouth disease, septic sore throat, etc. Many cases of sore throat are traced directly to streptococci in milk.

The method used to determine the number of bacteria in milk is similar to that used in water (see chapter under that heading).

CHAPTER XXIX

GENERAL CARE OF THE LABORATORY

Cleanliness should be the watchword. The laboratory should be cleaned at regular intervals; the floors and working desks should be cleaned once a week with a 5 per cent carbolic acid solution; all infected culture should be properly disinfected. In case of an accident—such as breaking a culture tube—a towel soaked in carbolic acid should be applied for an hour to the infected area, before the broken glass is removed and the place is cleaned. The desk should always be "cleared" at the end of the day's work. The workers should wear gowns while in the laboratory and should change them before leaving. They should carefully disinfect the hands after working with infectious material.

All stock cultures should be transferred at regular intervals varying according to their nature; staphylococci, typhoid-colon-dysentery group and other vigorous organisms may be transferred every month, but had better be kept in the ice box, as should be pneumococci, which, however, must be transferred more frequently. Animals, usually rabbits and guinea pigs, should be kept in clean places, fed oats, cabbage, and water, and the cages should be cleaned daily.

CHAPTER XXX

LIST OF QUESTIONS

1. Name the most important discoveries in bacteriology.

2. Name five important discoveries made by American bacteriologists.

3. What are bacteria? What is their structure? Their shape?

4. What is a flagellum? A spore? A capsule?

5. How are bacteria reproduced? Their chemical composition? Their food requirements?

6. What is a parasite? Saprophyte? Symbiosis? Antagonism? Give examples of each.

7. How does temperature affect the bacteria? Moisture? Drying?

8. Is capsule formation constant in the same species? Significance of capsule? Of spores?

9. What do bacteria do? Discuss fully, mentioning their various activities.

10. Of what use are bacteria in nature and in animal body.

11. What is the effect of electricity, radium, x-rays, sunlight, and heat on bacteria?

12. How should sputum, urine, and feces be disinfected? The room? The patient at recovery? The nurse? Apply this to a case of typhoid fever.

13. What are the different methods of destruction of bacteria? Their various purposes?

14. What is aerobe, anaerobe, either obligatory or facultative?

15. How are bacteria studied? What are the most commonly used stains? What is Gram's method of staining?

16. Name ten Gram-negative and ten Gram-positive bacteria.

17. What is a culture medium? What is an "ordinary" medium and what is an "enriched" medium. Name three of each, stating for what organism they may be used.

18. What is a "hanging drop" preparation and what is it used for?

19. What are some of the usual bacteriologic examinations? What is examined and for what infection?

20. Name three anaerobes and explain how they may be cultivated.

21. What is infection? Immunity? Its varieties?

22. What is necessary for an infection? What is virulence? Antigen? Antibody?

23. What is Ehrlich's theory of immunity? Metchnikoff's?

24. How do bacteria produce an infection? What is exotoxin, endotoxin, antitoxin? Blood serum?

25. What is a vaccine? In connection with what diseases is it used? How is it prepared?

26. What is an amboceptor? Agglutinin? Precipitin? Bacteriolysin? Hemolysin? Hemolysis? Phagocytosis? Opsonin? Leucocytosis? Complement? Weigert's law?

27. How is antitoxin serum prepared? In what diseases is it used? Widal test?

28. What is a Wassermann test? Anaphylaxis?

29. For what is blood most often examined? Sputum? Urine?

30. What diseases are produced by staphylococci and streptococci? How do they differ from each other mor-

phologically, culturally, and in disease-producing properties?

31. What bacteria cause pneumonia? Describe briefly pneumococci. What is the latest work on pneumococci?

32. Describe briefly gonococci and meningococci. Of what value is the immunity treatment in infections with staphylococci, streptococci pneumococci, and meningococci?

33. What are the members of the typhoid group? How do they resemble each other and how can they be differentiated?

34. Describe the tetanus bacillus, gas bacillus.

35. Describe the tubercle bacillus.

36. Describe the diphtheria bacillus. What other bacteria belong to the "acid-fast" group?

37. Describe the organism causing influenza, whooping cough. Describe plague bacilli.

38. Describe the anthrax bacillus.

39. Describe the cholera spirillum.

40. What diseases are caused by spirochetes? Describe the most important one.

41. What diseases are caused by yeasts and molds?

42. Describe malaria and its parasite.

43. What diseases are caused by "higher bacteria"?

44. What are the "diseases of unknown causation"?

45. What is Pasteur treatment?

46. Why is the knowledge of bacteriology important to the nurse?

47. What bacteria are of importance in air? Soil? Water? Milk?

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